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Resumen por el autor, Francis L. Landacre.

El destino de la cresta neural en la cabeza de los Urodelos.

La cresta neural de *Plethodon* se incorpora primeramente al tubo neural, y después emigra desde la superficie dorsal del tubo en dirección ventral, a lo largo de la superficie lateral del tubo, formando porciones de los ganglios de los nervios V, VII, IX y X, continuando después su emigración ventral hacia las barras branquiales, arco mandibular y arco hioideo, en los cuales forma mesenquima y cartílagos. El mesenquima ectodérmico puede distinguirse del derivado del mesodermo (mesenquima endodérmico) por el tamaño de las células, número y tamaño de los granos de vitelo, pigmentación y por la continuidad de las láminas de células emigrantes. El mesodermo produce todos los músculos de la cabeza, mesenquima de la región cefálica dorsal, y la porción posterior de las trabéculas, los paracordios, base del cráneo, arco occipital, cápsula auditiva y segundo basibranquial. La cresta neural produce la porción anterior de las trabéculas, cartílago de Meckel, palatoc cuadrado, y todos los cartílagos branquiales con la excepción del segundo basibranquial, así como el mesenquima de la región ventral de la cabeza.

El ectodermo lateral de la región oral produce un collar ectodérmico, en el cual se desarrollan los dientes y tejidos adyacentes. Con la excepción del collar ectodérmico oral, el ectodermo lateral no produce mesenquima, sino las placodas dorso-laterales, placodas epibranquiales, parte de los ganglios de la línea lateral, líneas laterales y ganglio del nervio profundo.

THE FATE OF THE NEURAL CREST IN THE HEAD OF THE URODELES

F. L. LANDACRE

Department of Anatomy, Ohio State University

ELEVEN FIGURES

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INTRODUCTION

During the course of an investigation of the origin of the cerebral ganglia of the urodeles it became necessary to determine accurately the ventral limits to which the neural crest migrates. In the attempt to do this the distribution of the neural crest was found to be so extensive and to involve so much tissue of a non-nervous character that it was decided for the time being to discontinue the study of the cerebral ganglia and follow the history of that portion of the crest which is not concerned in the formation of these ganglia.

The ventral limit of ganglia into the composition of which the neural crest enters, namely, V, VII, IX, and X, is at the level of the epibranchial placodes, approximately at the dorsal border of the corresponding gill slit. The neural crest in all vertebrate types, apparently, migrates ventral to this level beyond its ganglion-forming region and further is distributed in the head of the embryo anterior to the level of the gasserian ganglion.

Only a relatively small portion of the neural crest in the head is involved in the formation of cerebral ganglia.

In the urodeles the neural crest forms in the anterior head region an almost continuous sheet of mesenchyme, lying lateral to the axial mesoderm which is derived from the endoderm, and is interrupted for a time by the olfactory bulb and optic stalks only. In the region of the mandibular and branchial bars, the neural crest migrates ventrally into these bars to the extreme ventral level of the body and is prevented from forming a junction with the crest of the opposite side by the presence of the heart and ventral aorta.

The possibility of determining the fate of neural crest cells that do not enter into the composition of cerebral ganglia depends upon conditions that exist in some types and are apparently absent in others. It is the almost unanimous opinion of workers that it is extremely difficult and often impossible to determine the fate of all neural crest cells in the anterior head region. In the branchial region of the lower vertebrates particularly, the situation is very different. A number of reliable workers have given detailed descriptions of the fate of the neural crest cells and of the manner in which they enter into the composition of structures usually considered as mesodermal in origin. As to the migration of the neural crest beyond ganglion-forming regions in all vertebrates, there is apparently little doubt.

In the urodeles the determination of the extent of the migration of the neural crest and the differentiation of the neural crest and its derivatives from those of the endoderm are comparatively easy except in the anterior region. The contrast between the large, heavily yolk-laden, light staining endoderm cells and their derivatives on one hand and the smaller, dark staining, pigmented, slightly yolk-laden ectoderm cells and their derivatives on the other hand is very striking. In addition to these distinctions based upon size of cells, staining reaction, pigmentation, and number of granules, there is also an actual difference in the size of yolk granules carried by endoderm cells as compared with those carried by ectoderm cells, those carried by endoderm cells being larger. These distinctions, furthermore,

persist up to so late a stage in development in certain regions of the head that it can be determined accurately that head structures, usually considered as of mesodermal origin, are derived from the ectoderm of the neural crest.

HISTORICAL SKETCH

The idea that ectodermal cells, derived from either neural crest or from the lateral ectoderm, can be traced into permanent head structures, other than ganglia, was first stated definitely by Miss Platt in 1893. It was claimed in a preliminary paper published by her at that time that branchial cartilages arose from ectodermal cells. Before the appearance of her paper, however, several papers appeared bearing more or less directly on the fate of the non-nervous portion of the neural crest. Marshall ('78), working on the chick, and van Wijhe ('82), on the selachians, both called attention to the fact that the neural crest is present in the head anterior to the trigeminus ganglion, but could not determine the fate of that portion of the crest which does not enter into the formation of that ganglion.

Kastschenko ('88) went a step farther and stated that, in the head of the selachian embryo, other layers than the endoderm, particularly the ectoblast, take part in the formation of mesenchyme, and that in the formation of the neural crest some of the cells detach themselves from the neural-crest mass, become loosely arranged, and form mesenchyme, while other cells from the same source are concerned in the formation of ganglia. The further fate of the mesenchymal cells is not discussed, neither is their relation to mesenchyme derived from endoderm treated.

Goronowitsch ('92) published a preliminary paper in which he determined for birds not only that the neural-crest cells give rise to mesenchyme in the head region, but that they fuse with the axial mesoderm derived from the endoderm to form a homogeneous mesenchyme in which neural-crest cells can no longer be distinguished. He also noted a proliferation of mesoderm (mesenchyme?) cells from the ectoderm in the dorsal portion of the gill.

Miss Platt ('93), as noted above, published a preliminary notice in which she traced the branchial cartilages definitely to the ectoderm derived apparently from the lateral body wall rather than from the neural crest. Her statement is rather hard to follow and caused her work to be criticised and possibly to be misunderstood. She seems to derive the mesoderm from two longitudinal ridges which later break up into three vertical ridges. The median portion of each vertical ridge proliferates mesoderm into the gill arch to form the cartilage of the gill arch. Ventral to this point the lateral ectoderm proliferates cells into the branchial arch to form mesoderm. Miss Platt states that the neural cells (neural crest?) break up into stellate mesoderm, whose fate she is unable to follow. She does not in her preliminary paper introduce the terms 'mesectoderm' and 'mesentoderm' for mesenchyme derived, respectively, from ectoderm and endoderm.

Later in the same year Goronowitsch ('93 a and b) published on the development of the neural crest in birds and fishes and asserted again the origin of mesenchyme from both the neural crest and the lateral ectoderm. He makes the surprising statement that the neural crest is concerned neither in the formation of ganglia nor nerves, but only in the formation of mesenchyme, a part of which latter forms the sheath of Schwann and determines the course of growing nerves. The nerves arise from neuroblasts. Goronowitsch derives mesenchyme from the ectoderm in the region of the gill arch. The periaxial mesoderm comes from both neural crest and from the lateral ectoderm and is associated with the formation of the gill arch presumably, but he does not derive the cartilages specifically from the ectoderm as Miss Platt had done.

In 1894 Miss Platt elaborated the idea contained in her preliminary paper introducing the terms mesectoderm and mesentoderm for mesenchyme derived from the ectoderm and endoderm, respectively. She further recognizes the neural crest as contributing to the formation of mesectoderm, though the lateral ectoderm is its chief source. The axial mesoderm is recognized in this paper as coming from the endoderm, but in later papers

(Platt, '96, '97) this is questioned, and as a consequence she discards the term mesentoderm. In these later papers she derives ganglia, nerves, mesenchyme, and branchial cartilages and the dentine of the teeth from the mesectoderm, but neither muscles nor embryonic nerve supporting tissue (sheath of Schwann). In Miss Platt's second paper ('94) she follows the ectoderm cells into the gill bars, but not to their complete differentiation into cartilage. The later history of these cells and their differentiation into cartilages was given in the paper published in 1897.

Later papers by Kupffer ('95), by Lundborg ('99), by Dohrn ('02), and by Brauer ('04) support in the main Miss Platt's contention. However, Rabl ('94), Corning ('99), Minot ('01), and Buchs ('02) do not agree with her interpretation. The criticisms of Miss Platt's theory will be given first.

Rabl ('94) at the Strassburg meeting of the Anatomische Gesellschaft criticised Goronowitsch's description of the mode of derivation of the mesenchyme from ectoderm in birds and teleosts as an assumption, because Goronowitsch admitted that after ectoderm cells fuse with mesoderm he could no longer follow them, and consequently he had no right to assume that they became a permanent part of the mesenchyme. This objection seems to be valid so far as birds and bony fishes are concerned. His objection to Miss Platt's mode of derivation of cartilage is based not on a study of *Necturus*, which he admits he has not examined, but on a study of *Triton*, salamander, and axolotl. He thinks that the appearance, which Miss Platt finds in her preparations, of cells being proliferated from the ectoderm, can be explained best as due to faulty fixation. Rabl makes a vigorous defense of the idea of the integrity of the germ layers. Most of his criticism is devoted to Klaatch's ('94) conception of the origin of the skeleton of the fins of the fishes. Harrison ('95) has since shown that Klaatch was wrong in his interpretation.

Corning ('99) derives, in the *Anura*, from the neural crest, only ganglia and nerves, the ventral portions of which extend well down into the corresponding branchial arches. The neural crest is closely fused with the lateral ectoderm, deriving some of

its cells from that source, but in Rana it does not break down into mesenchyme, according to this author. The mesenchyme of the head is derived from the endoderm, and is consequently mesentoderm in Miss Platt's sense of this word or mesoderm in the older sense.

Minot ('01), in an address before the New York Pathological Society, makes a vigorous defense of the doctrine of the integrity of the germ layers, in which he agrees with Rabl ('94), whose opinion was expressed under somewhat similar circumstances. He calls attention to Miss Platt's work specifically and says, "an examination of a number of series and stages has not enabled me to find the slightest evidence in favor of Miss Platt's conclusions." He says further that "we may, therefore, I think safely regard this attempt to overthrow the morphological value of the germ layers as unsuccessful. I know of no other attempt of sufficient importance to be even mentioned." He states in an earlier paragraph that "the efforts to upset the validity of this fundamental doctrine have failed to find support or recognition from any leading embryologist." These statements of Doctor Minot's were made previous to the appearance of the work by (Dohrn ('02) and Brauer ('04), but after the appearance of that of Kupffer ('95) which he must have overlooked. Both Minot and Rabl seem, in the opinion of the writer, to have given too much weight to the doctrine of the integrity of the germ layers in their estimate of a question, which is purely one of accuracy of observation and description.

Buchs ('02), after studying Necturus, disagrees with Miss Platt's conclusions, taking exception particularly to her statements concerning the derivation of mesenchyme from the lateral ectoderm and to her distinction between mesectoderm and mesentoderm on the basis of the amount and size of the yolk granules. Buchs' opposition to Miss Platt's interpretation of the mesenchyme is based frequently on minor details and possible ambiguities in statements, although he has worked over the same type. He derives cartilage from mesenchyme which arises from endoderm by a folding process of the endoderm and not from ectoderm. He can find no evidence for the wandering of

the precartilag cells from the nerve anlagen or from the ectoderm. He denies the contribution of cells from the ectoderm to the mesenchyme, but does not follow the fate of neural-crest cells.

Of the writers opposing Miss Platt's hypothesis, two only, Corning ('99) and Buchs ('02), give sufficiently detailed descriptions and figures to enable one to estimate the value of their criticism. Corning certainly did not follow his stages far enough to determine that the mesoderm of the branchial bars is completely surrounded by neural-crest cells. This can be seen on any good series of the frog, *Rana pipiens*. Buchs, on the other hand, contents himself with an effort at destructive criticism. His actual evidence is of a negative character, since he does not follow the fate of neural-crest cells that do not form ganglia.

Of the authors who support wholly or in part Miss Platt's contention, the earliest is Kupffer. Kupffer ('95) described in detail and figured the cartilages in *Ammocoetes* as arising from the deeper layer of the ectoderm in the region of the branchial bars. He had previously ('94) designated this layer as neurodermis, believing it to be concerned in the formation of the branchial nerves, but here designates it as branchiodermis and derives not only the cartilages, but goes a step farther than Miss Platt and derives muscles also from it. He agrees fully with Miss Platt's interpretation after examining her preparations. He further derives mesenchyme in the dorsal anterior head regions from the neural crest.

Lundborg ('99) derived the pterygopalatine cartilages in *Salmo salar* and the trabeculae in *Rana temporaria* from the ectoderm of the roof of the mouth. He also derived the ethmoid cartilages in *Salmo* in the same manner. The anterior end of these cartilages in *Salmo* are in process of formation in sixty-eight-day-old embryos.

Koltzoff ('02), in *Petromyzon*, derives mesenchyme in the head from both the neural crest and lateral ectoderm, but is unable to follow its fate beyond the point of the mingling of ectodermal cells with those derived from endoderm.

Dohrn ('02) gives a full description with numerous figures of the migration of the neural crest ventrally into the branchial region and its metamorphosis into mesenchyme and cartilage of the branchial bars in *Torpedo ocellata*. He agrees fully with Miss Platt's interpretation of the origin of branchial cartilages from ectoderm, although he derives them from the neural crest rather than from the lateral ectoderm. He does not exclude the contribution of the cells from the lateral ectoderm in later stages, although neither his figures nor text include such a contribution. He adopts Miss Platt's term *mesectoderm* and criticises the effort of Corning to maintain the integrity of the germ layers in the formation particularly of structures derived from the mesoderm. Dohrn's evidence for the derivation of connective tissues and cartilage from ectoderm is more convincing, if possible, than that of Brauer in the *Gymnophiona* and of Kupffer in *Petromyzon*, both of whom give detailed descriptions. Dohrn's figures taken with Neal's ('98) reconstructions of *Squalus* (figs. 7 to 21, pls. 3 and 4) furnish convincing evidence for the continuous migration ventrally (Neal) of the neural crest and its ultimate transformation into permanent mesenchyme and cartilage of the branchial bars (Dohrn).

Brauer ('04), working on the *Gymnophiona*, derives mesenchyme of the anterior region of the head from neural-crest cells which later mingle with cells derived from endoderm to form mesenchyme in which the two derivatives cannot be recognized after this fusion. He can find no evidence for the disappearance of these neural-crest cells, however, before their fusion with cells derived from the endoderm. In the posterior head region neural-crest cells, when not involved in the formation of ganglia, grow ventrally into the branchial bars and surround the mesoderm of the bar at first lying on its lateral surface, but finally entirely surrounding it. He can find no evidence for the derivation of mesenchyme in the gill bar from the adjacent lateral ectoderm, as Miss Platt had done, but derives it entirely from the neural crest. He does not deny the possible later mingling of cells derived from mesoderm with those derived from the neural crest, but insists that the chief part in the formation of

the gill bar is performed by cells derived from the neural crest. Brauer objects to Miss Platt's terms mesectoderm and mesentoderm which had been accepted by Dohrn and Koltzoff. He reserves his description of the ultimate fate of the ectodermal mesenchyme of the gill bar for a later paper and consequently does not describe the origin of definite cartilage.

An impartial examination of the papers cited above furnishes strong evidence for the formation of the mesenchyme in the anterior head region in the embryo from both endodermal (mesentoderm) and ectodermal (mesectoderm) sources. In all cases the fate of these individual cells is lost and it is not possible to determine the extent to which either or both of them is concerned in the formation of adult mesenchyme in this region. However, evidence for the disappearance of mesectoderm cells in the anterior head region is conspicuously absent. The same statement holds in the main for the dorsal mesenchyme in the posterior portion of the head.

The fate of the ectodermal derivatives in the branchial regions is much more definitely stated. Platt ('93), Kupffer ('95), Brauer ('04), and Dohrn ('02), all derive either the cartilages and mesenchyme or both cartilages and muscles in addition to mesenchyme from the ectoderm. Platt ('93), Kupffer ('95), and Koltzoff ('02) derive the ectodermal cells in the branchial region largely or even altogether from the lateral ectoderm, while Brauer ('04), Corning ('99), and Buchs ('12) can find no evidence for the proliferation of cells from the ectoderm, and Brauer ('04) and Dohrn ('02) derive the structures in the branchial bar entirely so far as they are ectodermic from the neural crest.

MATERIAL

The material on which the work was done consists of three series of urodele embryos collected in the same pond, but representing at least two different species. The youngest series (series I) consists of thirty-five stages taken from two egg clusters at intervals of five to five and one-half hours, reared at room temperature, and covers an interval of eight days. The first

stage of this series was fixed immediately after collection and the medullary folds appear in stage 6. This series, judged by the size of the egg clusters and character of development, is evidently *Plethodon glutinosus*.

The second series is older and consists of thirty-two stages taken from four egg clusters reared at room temperature and fixed at intervals of about five hours. The total period covered by this series is about eight days. The youngest stage of this series corresponds closely to no. 32 of series I. The larvae were reared and identified as *Plethodon glutinosus*. These larvae hatched in the twenty-fourth stage, so that combining series I and II there are fifty-six stages taken previous to hatching.

The third series (series III) consists of fifty-one stages taken from five egg clusters reared at room temperature and fixed at four-hour intervals. The total period covered is again about eight days. The first stage of series III corresponds closely to no. 10 of series I and the twenty-third stage of series III corresponds to no. 1 of series II. The larvae of this series were reared up to metamorphosis, but escaped before an identification could be made. Seventeen stages of this series only were cut.

Including duplicates, there are 292 slides in all. All stages were cut transversely, and critical stages were cut in the sagittal and coronal planes also. The material was fixed in Zenker and stained in Delafield's haematoxylin and counterstained in orange G. The younger stages were covered with a film of celloidin to prevent the loss of yolk-laden cells which are likely to become detached. The closeness of the series and their sequence are particularly important in the discussion of the problem involved in this paper. Where a series is taken from more than one cluster of eggs, care was used to insure that the series would be continuous by having the eggs taken from the two lots overlap in time.

THE ORIGIN OF THE NEURAL CREST

There are in the vertebrates three modes in which the neural crest is related to the neural tube and overlying ectoderm. Harrison ('01) has described and compared two of these. In the first type, the neural-crest cells—selachians and other types—represent the dorsal portion of the lateral walls of the neural tube which is at first continuous with the ectoderm. The neural crest is incorporated in the neural tube, forming a wedge-shaped mass in its dorsal portion. This wedge-shaped mass later becomes detached from the tube and migrates laterally and ventrally. In the second mode the neural crest (teleosts) lies between the dorsal border of the neural tube and the ectoderm, not being included strictly in either, but forming later a cap over the dorsal border of the neural tube. In the third mode (Ameiurus and other types (Landacre, '10)) the neural crest or cells homologous to the neural-crest cells remain in the ectoderm lateral to the neural canal and are later detached from this position to form parts of cranial ganglia and other structures.

The three series of urodeles studied correspond closely in the behavior of the neural crest to the first type mentioned above and do not require an extended description. The neural crest is completely incorporated in the dorsal border of the neural tube, but can be distinguished from the tube, usually, by its looser structure. The following description is based on the behavior of the crest at the level of the VII ganglion.

When the medullary plate can be identified first, it is very broad and its lateral borders include the two portions of the neural crest. Just before the closure of the neural canal (fig. 1) the superficial layer of the ectoderm and much of what will become neural crest are invaginated and included within the limits of the neural groove. The superficial heavily pigmented layer of the ectoderm forms the inner lining of the greater portion of the neural groove. The looser texture and greater pigmentation of the dorsomesial portions of the walls of the neural groove indicate roughly the position of the neural-crest cells.

In the closure of the neural groove (fig. 2) the superficial pigmented cells lining the dorsal two-thirds of the neural tube come into contact and obliterate that portion of the canal lined by flat cells. The line of juncture is indicated by the heavy pigmentation of the cells. The dorsomesial portion of the wall of the tube has a looser texture and less of a syncytial character than the lateral and ventral portions. The neural tube as a whole is well delimited from the ectoderm except at the dorsal border.

The outline of the neural tube (fig. 3) as distinct from the neural crest first becomes apparent when the cells of the tube assume a syncytial character with their nuclei arranged with their long axes toward the center of the neural canal. At the same time the neural crest while still forming a conspicuous wedge in the dorsal portion of the tube is evidently now largely outside the limits of the tube. The dorsal third of the tube becomes neural crest and presents the appearance of being erupted from the tube. The tube is horseshoe-shaped with the open dorsal portion filled with a wedge of neural-crest cells. The tube except at the ventral border is of uniform thickness.

The next step (fig. 4) involves the further exclusion of neural-crest cells from the dorsal wall of the tube. In this process many of the loosely arranged, heavily pigmented cells are left for a time in the position of the original wedge. In fact, after the neural crest is well defined and has begun to migrate ventrally (fig. 5) a few cells of this type still form the roof plate of the neural canal. In this stage (fig. 5) the greater portion of the neural crest rests upon the dorsal portion of the neural tube, but there are two prominent lateral extensions lying between the dorsolateral border of the neural tube and the ectoderm. At a slightly later stage (fig. 6) the neural crest is represented almost exclusively by these lateral extensions. The large mass of cells previously lying over the tube is now represented by a few flat cells connecting the two lateral portions of the neural crest. These flat cells disappear later. In figure 6 the original wedge seems to be represented by a few irregularly arranged cells.

Up to the last stage described there is practically no indication that cells are added to the crest, as defined, from the lateral ectoderm. The crest is continuous on its dorsal border with the inner layer of the ectoderm, and undoubtedly receives cells from this source (figs. 1, 2, 3, and 5), and in several series at the age of that from which figure 3 is taken and the crest is continuous ventrally with the inner layer (fig. 3). This continuity is usually absent at the level under consideration as the lateral portions of the crest become better defined (figs. 5 and 6) and also in the earlier stages (fig. 4).

These conditions as described at the level of the VII ganglion are duplicated at the levels of ganglia V, IX, and X. In the intervals between these ganglia the neural-crest cells are less numerous and the lateral extensions contain fewer cells and do not reach so far ventrally. At the stage from which figure 5 was taken (fig. 7), the neural crest is continuous throughout the whole region. In the stage from which figure 6 was taken (fig. 8) the crest is interrupted, except for a few scattered cells on the dorsal portion of the cord, between the V and VII ganglia and again between the VII and IX ganglia.

THE MIGRATION OF THE NEURAL CREST AND ITS RELATION TO THE MESODERM

The accuracy with which the migration of the neural crest can be followed depends upon histological differences between cells derived from the ectoderm as distinguished from those derived from the endoderm. These distinctions have been stated already in the introduction (p. 2). They will be assumed for the present and the migration of the crest will be described from the reconstructions given in figures 7 to 11. The discussion of the basis of these distinctions together with the discussion of two other disputed points, namely, the question as to the contribution by the lateral ectoderm of cells to the mesenchyme and the question as to the fate of cells in the anterior head region, will be deferred to a later section.

As to the use of terms, Miss Platt rejected the term 'mesenterm' presumably because the term implies a derm or germ layer

derived from entoderm, and of course this is equivalent to mesoderm and therefore superfluous. There is in the head region, however, a good deal of loose tissue quite similar to mesenchyme found in the trunk and like that found in the trunk, derived from mesoderm, which is itself a derivative of endoderm. Now, since it is necessary, in describing the behavior of mesenchyme in the head, to distinguish between that derived from the neural crest, which retains most of its ectodermal characters, and that derived from mesoderm, which for a long time retains its endodermal characters, I have ventured to suggest the terms 'entodermal mesenchyme' and 'ectodermal mesenchyme' for mesenchyme in the head where the two types of cells can be distinguished. When head mesenchyme becomes homogeneous, that is when we can no longer distinguish two types of cells, it will be referred to as mesenchyme, with the implication, however, that it sometimes contains both ectodermal and entodermal cells. While both types of mesenchyme have passed through intermediate stages, the entoderm through a mesodermal stage and the ectoderm through a neural crest stage, each retains the character of its more remote rather than of its immediate ancestor. This seems to justify the terms ectodermal mesenchyme and entodermal mesenchyme rather than neural-crest mesenchyme and mesodermal mesenchyme. The term ectodermal mesenchyme is substituted for mesectoderm, which has become general in the literature. The tissue we are dealing with is not a derm or layer, but a true mesenchyme quite similar to mesenchyme in the trunk, but coming from ectoderm rather than from mesoderm as is in the trunk.

The use of the terms ectodermal mesenchyme and entodermal mesenchyme is further justified by the fact that in the head all branchial muscles come from the mesoderm, and show throughout their earlier stages definite somatic and splanchnic layers, indicating their relation to the lateral mesoderm of the body, while the mesenchyme of the head is more or less loose and of a syncytial character, like that derived in the body from the sclerotomes and the lateral and splanchnic mesoderm. As the mesoderm of the anterior trunk region grows forward into the

head, it gives rise to *a*) eye muscles from its dorsal or somitic portion; *b*) branchial muscles from its ventral portion, and, *c*) mesenchyme which retains its endodermal characters. That portion of head mesoderm which gives rise to branchial muscles does not become loose or syncytial in character as does the mesenchyme, but retains its integrity to such an extent that two layers, somatic and splanchnic, can for a long time be identified.

The neural crest, on the other hand, gives rise to specific cerebral ganglia such as V, VII, IX, and X and then migrates ventrally into the ventral head region and into the branchial bars and differentiates into cartilages and loose mesenchyme. The neural-crest ectoderm furnishes three specific derivatives, *a*) ganglia, *b*) cartilages, *c*) mesenchyme. Since the two types of mesenchyme overlap and are histologically distinct for a long time and since the ventral portion of the neural crest passes from a mesenchymal stage to a cartilaginous stage, the terms entodermal mesenchyme and ectodermal mesenchyme seem not only to be justified, but to be absolutely necessary to accurate description.

We shall first follow the migration of the neural crest and mesoderm.

In the first stage plotted, which is 3 mm. in length (fig. 7), the neural crest is continuous along its dorsal border throughout the whole head region, beginning anteriorly at the level of the middle of the eye horizontally and extending caudally into the spinal-neural crest. It presents three conspicuous enlargements. The anterior enlargement extends caudally to the vertical level of the posterior border of the eye. The gasserian ganglion differentiates out of the posterior portion of this enlargement and can be identified at this stage by the slight condensation of the cells. This enlargement is a ventral extension of the neural crest, but owing to the flexure of the head it seems to extend caudally.

The second enlargement or ventral extension is at the level of the VII ganglion, the third at the level of the IX ganglion, and the fourth inconspicuous enlargement at the level of the

anterior division of the X ganglion. Posterior to this level the neural crest gradually becomes narrower dorsoventrally and passes into the neural crest of the spinal cord. In referring to the V, VII, IX, and X ganglia it is to be understood that only the general cutaneous and general visceral portions of these ganglia are under consideration. These are homologous to spinal ganglia. The special somatic and special visceral ganglia are referable to other sources.

The endodermal derivatives at this stage fall into two regions. First the posterior head region, which extends cephalad to the level of the anterior end of the alimentary canal and is characterized by the presence of somites and a two-layered lateral mesoderm. The somites are not well defined at this stage and the lateral mesoderm forms a broad sheet extending from a level slightly dorsal to the notochord to the ventral limit of the body, being absent only in the heart region and the region of the stomodaeum.

The second, or anterior head region, lies anterior to the level of the anterior end of the alimentary canal and forms a loose mesial or axial mass of entodermal mesenchyme and two lateral extensions. These lateral extensions contain cavities which in the urodeles do not form definite head cavities or somites, but their dorsal portions are undoubtedly homologous to the head somites of selachians. The dorsal border of this entodermal mesenchyme maintains the same relative level as the dorsal border of the somites. It reaches cephalad to the vertical level of the middle of the optic vesicle and ventrally it forms a prominent extension, which is separated from the anterior border of the lateral mesoderm by an area free from mesoderm cells. This area corresponds roughly to the future position of the spiracular gill cleft. The ventral border of this extension reaches nearly to the ventral limit of the optic vesicle. The whole of this anterior head mesoderm consists of the large endoderm cells containing large yolk granules and except those regions giving rise to eye and branchial muscle has a loose arrangement presaging its modification into typical mesenchyme. The neural crest overlaps the cells derived from endoderm at two points only—

in the region of the V ganglion and at the ventral border of the IX ganglion. In the overlapping areas mentioned the entodermal mesoderm lies mesial to the neural crest.

In the second stage plotted, 3 mm. in length (fig. 8), but six hours older than the last, the neural crest in the region of the gasserian ganglion and anterior to this ganglion has migrated ventrally and caudally and now forms two prominent extensions, the one anterior to the eye and mesial to the olfactory capsule, the other posterior to the eye and extending slightly into the mandibular bar ventral to its ganglion-forming region. This anterior neural crest is now completely detached from the neural crest in the region of the VII ganglion, except for a few scattered cells on the dorsal portion of the neural tube.

The neural crest at the level of the VII ganglion has grown ventrally to the region of the dorsal border of the alimentary canal, doubling its length as compared with the last stage. The IX ganglion has also grown ventrally to the same extent and the X is now represented by a conspicuous ventrocaudal extension of the same general neural-crest mass from which the IX ganglion forms. The neural crest of VII, IX, and X have not, as yet, grown ventrally beyond their ganglion-forming regions. The neural crest in the region of VII is connected dorsally by a well-defined strand of neural crest cells with that of ganglia IX and X.

The lateral mesoderm lying posterior to the anterior end of the alimentary canal at this stage is interrupted by the outgrowth of the endoderm to form the spiracular and hyoid pharyngeal pouches. The interruption of the lateral mesoderm at the level of the spiracular cleft is not formed entirely by the pharyngeal pocket. Its ventral portion represents the remains of the prominent notch shown just caudal to the eye in figure 7.

The head mesoderm lying anterior to the alimentary canal has extended in two directions, dorsally and anteriorly, until it has reached the dorsal wall of the brain at the vertical level of the anterior end of the optic vesicle. It has also extended ventrally and caudally posterior to the optic vesicle. The posterior boundary of this extension lies just ventral to the spiracular pharyngeal pocket. The ventral limit of this extension

reaches almost to the ventral border of the optic vesicle. The region of the overlapping of neural crest and mesoderm is quite extensive, as indicated in figure 8. In the region just posterior to the eye the mesoderm is not completely covered by neural crest. In the region of the VII, IX, and X ganglion the ventral half of each ganglion overlaps the lateral mesoderm and lies lateral to it.

In figure 9 from a stage 4 mm. long and ten hours older than the stage from which figure 8 was taken, the neural crest has not altered its relation greatly except that posterior to the eye it has grown ventrally and caudally into the mandibular bar and now almost covers, on the lateral surface, the primordium of the mandibular muscles. This extension reaches almost to the ventral limit of the body and far beyond the ventral limit of the V ganglion. In the region of the VII and X ganglia, the ventral extensions of the crest have moved into the hyoid and second true branchial bars reaching, at least in the case of VII, to the middle of the body and well beyond the ganglion-forming region. The crest in the region of IX shows little change.

The lateral mesoderm in the pharyngeal region is now interrupted by three visceral pouches, but is otherwise unmodified so far as its extent is concerned. In the anterior head region the mesoderm has extended cephalad over the eye and ventrally at the same level so that now it lies between the dorsal border of the optic vesicle and the brain. The increase in the amount of overlap of neural crest is most marked in the regions of the VII and X ganglia.

Figure 10 is taken from stage 12 of series III and is $4\frac{1}{2}$ mm. long and approximately twelve hours older than the stage from which figure 9 was taken. In this stage there are two striking changes in the extent of the neural crest noticeable in the anterior head region. The first is progressive and carries the neural crest ventrally and slightly cephalad in the region of the olfactory and optic vesicles. The olfactory capsule is now completely separated from the brain wall by a sheet of ectodermal cells which, extending ventral and caudal from the olfactory capsule, reaches the ventral limit of the brain wall below the optic vesicle.

Much of the optic vesicle, aside from the region of the optic stalk, is separated from the brain wall by this continuous sheet of ectodermal mesenchyme.

The second change in this region is regressive and is indicated by the absence of ectodermal mesenchyme in the region lying vertically over the olfactory capsule. The question as to whether this is due to a withdrawal by migration or to a disappearance of the cells will be taken up in the next section. Although the anterior limit of the ectodermal mesenchyme is hard to determine on account of the scattered neural-crest cells in this region, there is no doubt that the process of disappearance of neural crest at this point has taken place. It is much more marked in the next stage plotted (fig. 11). Posterior to the eye, the change in the extent of the ectodermal mesenchyme is slight as seen from the lateral surface. It is almost coextensive with the primordium of the mandibular muscle mass. It surrounds this mass completely, however, so that the endodermal derivative of the mandibular bar is inclosed by a sheet of ectodermal mesenchyme except at its extreme posterior tip.

In the posterior head region at the levels of the VII, IX, and X ganglia the most marked changes are the ventral growth of the neural crest into the hyoid and first two true branchial bars, with the accompanying process of surrounding the endodermal derivatives (branchial muscles) in these bars by ectodermal cells. This last process is, of course, not indicated on the plot. Posterior to the pharyngeal pouch of the second true gill in the region of X the neural crest has not only grown ventrally, but also caudally as a broad sheet which will be pierced by the third true branchial pouch, thus setting off the second branchial ganglion of X from the first. In this process the mode of development is altered somewhat, since in the more anterior gill bars the neural crest grows ventrally into the bars after they are formed by the pharyngeal endodermic evagination, while here the migration of the neural crest ventrally precedes the formation of the pharyngeal evagination and must be displaced by it. As in the more anterior ganglia, the dorsal portion of the broad mass extending caudally from X forms ganglion, while

the ventral portion takes part in the formation of the mesenchyme and cartilage of the gill bar.

In the last stage plotted (fig. 11) from stage 1 of series II, 6 mm. long, and approximately twenty-four hours older than that from which figure 10 was taken, the ectodermal mesenchyme has reached, except in the posterior pharyngeal region, its final distribution. Its later history involves its differentiation as distinct from its migration.

In the anterior head region, including the mandibular bar, the changes in distribution are both progressive and regressive. The most marked increase in extent of ectodermal mesenchyme in this region is in the mandibular bar due apparently to the growth of the bar. There is also a slight increase in the extreme anterior end of the head or rather a residue in the form of spur of ectodermal mesenchyme extending dorsally from the position of the epiphysis. This spur lies morphologically on the dorsal wall of the midbrain. In the region of the olfactory capsule the ectodermal mesenchyme forms a continuous sheet between the forebrain and ectoderm. It also forms a continuous sheet between the anterior border of the optic vesicle and ectoderm with a second spur extending dorsally to the level of the profundus ganglion and a third extending ventrally under the olfactory and optic capsules which is continuous with the ectodermal mesenchyme of the mandibular bar. Except for the first two spurs mentioned, the ectodermal mesenchyme is now confined as a continuous sheet to the anterior and ventral head regions anterior to the level of the gasserian ganglion. This represents a rather marked decrease in the relative extent of the ectodermal mesenchyme in the dorsal portion of the anterior head region.

In the posterior head region there is in this stage a marked ventral extension of the crest at the levels of V, VII, IX, and X ganglia. The ectodermal mesenchyme at these levels has grown into the corresponding gill bars and now reaches to the level of the heart and ventral aorta, covering from the lateral view the whole of the entodermal derivatives of these bars. Except in the posterior bar, the ectodermal mesenchyme has further completely surrounded the endodermal derivatives. Posterior

to the last pharyngeal pouch, the neural crest forms a broad sheet which will be perforated by the pharyngeal pocket of the third true gill. In this stage the neural-crest portion of the X ganglia possesses two ventral extensions or pharyngeal ganglia in addition to the third broad band lying behind the last pharyngeal pouch.

In the lateral mesoderm posterior to the anterior end of the alimentary canal there is no change except that it is perforated by an additional pharyngeal pouch. The withdrawal of the lateral mesoderm in the region of the last pharyngeal pouch is more extensive than the contact between the ectoderm and endoderm, due apparently to the fact that this pouch excludes both ectodermal mesenchyme and entodermal mesenchyme in reaching the ectoderm. Mesoderm is certainly absent over a much larger area after the contact is formed than is ectodermal mesenchyme. Anterior to the anterior end of the alimentary canal the entodermal mesenchyme occupies the whole head region except the area ventral to a line drawn from the region of the epiphysis to the base of the hypophysis. In this region at this stage the mesenchyme is derived from ectoderm. The area of overlap in the anterior head region is slight and can be best understood by reference to the reconstruction (fig. 11). In the posterior head region the ectodermal mesenchyme and mesoderm are coextensive except in the regions between the ganglia V, VII, IX, and X and in the region posterior to the X ganglia.

The general result of the migration of the neural crest may be stated as follows: it furnishes first the general somatic and general visceral portions of the V, VII, IX, and X ganglia. Whether it furnishes other portions of the V, VII, IX, and X ganglia must be deferred to a later paper. It also migrates into and furnishes mesenchyme in the early stages in the mandibular, hyoid, and pharyngeal gill bars. The mesenchyme at first lies lateral to the endodermal derivative which is always more dense in the bars, but later comes to completely surround it. The neural crest also furnishes a complete sheet of mesenchyme in the extreme ventral head region. The crest disappears as a

continuous sheet in the region of the midbrain except for the two dorsally directed spurs shown in figure 11 and between the V and VII ganglia, and as a continuous sheet disappears in the region immediately dorsal to the hypophysis. The identification of the crest in these regions rests on certain histological characters of its cells, but to some extent on the continuity of the sheet of cells which everywhere grows continuously in a ventral direction morphologically. The continuity of this sheet of ectodermal mesenchyme derived from the neural crest needs to be emphasized, since it facilitates greatly the identification of the progressive changes in extent of the crest. At points where the neural crest is diminishing in extent the problem of describing its boundaries is much more difficult.

The behavior of the head mesoderm, in addition to the facts shown by the plots, consists in the formation of branchial muscles from the lateral mesoderm, which do not lose their entodermal characters, the formation of eye muscles from the dorsal or somatic region of head mesoderm, and the formation of mesenchyme, particularly in the anterior and dorsal head regions, which mingles with detached cells derived from the neural crest to form later homogeneous head mesenchyme.

THE DERIVATIVES OF THE LATERAL ECTODERM

Among authors who derive mesenchyme from ectoderm, one of the most debated points is concerned with the origin of mesenchyme from the lateral surface ectoderm of the head as compared with the origin from the neural crest. Miss Platt, in her earlier paper ('94), derived ectodermal mesenchyme entirely from lateral ectoderm, but in her later paper seems to include the neural crest also a source of this tissue. Kupffer ('95) also, in *Petromyzon*, derives branchial cartilages and mesenchyme from the lateral ectoderm, which he designates as branchodermis, and later as neurodermis. Lundborg ('99) and Koltzoff ('02) both derive mesenchyme from lateral ectoderm. Dohrn ('02), however, working on *Torpedo ocellata*, and Brauer ('04), working on the *Gymnophiona*, can find no evidence that anything but

neural crest is concerned in the formation of ectodermal mesenchyme and cartilages.

This problem must be settled finally, of course, by observation; but since my results agree with those of Dohrn and Brauer, and since I have examined carefully types in which some authors derive mesenchyme from lateral surface ectoderm, it is interesting to examine conditions in the lateral ectoderm, which might have led to a misinterpretation of the facts by earlier authors.

The lateral ectoderm is concerned in the formation of a number of structures which do not form mesenchyme and their number and significance are impressive. These structures, taken approximately in the order of their sequence in development, are as follows:

a. In the early stages of the formation of the neural plate a few scattered cells lie between the lateral border of the neural plate and the thin surface ectoderm, but they are few in number and show every appearance of detached neural-crest cells. In the later migration ventrally of the neural crest they are either lost or incorporated with the neural crest. They certainly furnish no evidence for a general contribution of cells to the mesenchyme from the lateral ectoderm.

b. The profundus ganglion arises from the lateral ectoderm anterior to the gasserian ganglion and is quite extensive both in its longitudinal and its vertical diameters and shows at times the appearance of an extensive delamination. A careful study of its development shows, however, that the delamination of the ectoderm at this point is concerned solely with the formation of the profundus ganglion and not with mesenchyme.

c. The lateral ectoderm next gives rise to the lateral-line ganglion of nerves VII, IX, and X in certain types, and especially in those types in which the homologue of the neural crest is not included in the neural canal (type III, p. 11). These forms present the appearance of contributing cells to the mesenchyme. Their true fate, however, can be determined to be the formation of lateral-line ganglia, if one has a complete series taken at close intervals.

d. The lateral ectoderm also gives rise to the lateral-line primordia (placodes) which are distinct structures, although in some types closely related in time of appearance and sometimes even in position with other structures derived from the lateral ectoderm. If followed carefully, however, their identity can be established with certainty. Their thickenings or placodes are usually accompanied by detached cells and should not lead to confusion as to their fate.

e. Next the lateral ectoderm gives rise to the epibranchial placodes which furnish the special visceral or gustatory portions of the VII, IX, and X ganglia. Since these placodes become completely detached from the lateral ectoderm and added to the neural-crest portions of the VII, IX, and X ganglia and do not always have definite boundaries, they might be misinterpreted as contributions of the lateral ectoderm to mesenchyme in the branchial region. If these placodes are followed carefully they are found to have nothing to do with mesenchyme, but are ganglion-forming structures.

Aside from the oral region, which must be taken up more in detail, these five structures are all that might be mistaken for contributions to the mesenchyme. The point to be emphasized, aside from our own observation, is this: any claim that the lateral ectoderm contributes to the formation of mesenchyme that does not take into consideration and account for the structures mentioned above is open to serious criticism. We believe that the disagreement among authors as to the exact condition in the lateral ectoderm is due to failure to account for the structures enumerated above before assuming that cells delaminated from the lateral ectoderm went into the formation of mesenchyme.

With the facts mentioned above in mind, we have examined carefully our series and, aside from the oral region yet to be described, we can find no evidence for the contribution of lateral ectoderm cells to mesenchyme. In fact, the ectoderm at all points except those mentioned is intact and shows no indication of liberating cells, and we find ourselves in the closest accord with the statements of Dohrn ('02) and Brauer ('04), who can find no such contribution.

THE ORAL ECTODERM

Lastly, the oral region in the urodeles presents a curious condition with reference to the positions of ectoderm and endoderm. None of the authors interested primarily in the origin of head mesenchyme has described this region carefully. This is all the more striking, since, on superficial examination, it would seem to furnish the best evidence for the derivation of mesenchyme from the lateral ectoderm. Johnston ('10), working on the fate of the oral endoderm in *Amblystoma*, gives an accurate description of the oral region. He does not, however, follow the fate of the mesenchyme. My results agree with his, except in one particular.

I find, in agreement with Johnston, that the anterior end of the archenteron is open at first well forward to the position of the future oral opening. This open oral and pharyngeal cavity next becomes closed and forms a solid, flat column of endoderm abutting cephalad against the oral ectoderm, which at this point consists of the usual two layers, a superficial epithelium and a deeper layer of nervous ectoderm. The next change consists in the formation of a collar of ectoderm around the solid column of oral endoderm. This collar grows caudally from the anterior oral region and mesially from the lateral oral region as far as the position of the vomerine teeth, which is approximately the posterior boundary of the solid column of the oral endoderm. This ectodermic collar is derived from lateral ectoderm as distinct from neural crest. During the formation of the ectodermal collar the nervous layer of the ectoderm disappears at the line of the future opening, but we are unable to verify Johnston's statement that the ectoderm disappears entirely, leaving the endodermal column exposed at the oral region. In our preparations the surface ectoderm is never absent at this point before its rupture to form the oral opening.

The next change consists in the formation of a definite oral cavity in the previously solid column of oral endoderm. It splits from behind forward, and after the endodermal column splits, the thin layer of superficial ectoderm ruptures, thus forming

a true oral cavity lined entirely by entoderm, but possessing a collar of ectoderm, dorsal to its roof and ventral to its floor. This curious relation of the ectoderm to the entoderm is to be interpreted as a modification of the usual process of producing an oral cavity or stomodaeum by the invagination of ectoderm. In the urodele the solid column of oral endoderm which abuts against the ectoderm cephalad prevents the formation of the usual ectodermal invagination and we have the ingrowth of ectoderm to form a collar around a solid column of endoderm, which later splits to form an oral cavity lined by endoderm instead of by ectoderm as in other vertebrates. The ectoderm which in other vertebrates lines the oral cavity is in the urodeles, consequently, separated from the oral cavity by endoderm.

The ectodermal collar is the structure of greatest interest in the present discussion. The shape of the mouth in the urodeles is indicated during the solid stage of the oral endoderm by the great breadth of the endodermal column; consequently, the ectodermal collar is also broad and appears in transverse sections as broad plates of ectoderm lying dorsal and ventral to the endodermal column. These plates are curled at the lateral borders of the solid endodermal column, bending dorsolaterally and ventrolaterally around the lateral borders of the endodermal column and projecting slightly between what will be the roof and the floor of the oral cavity. These infolded edges cover the future lips of the oral cavity laterally with ectoderm. The ingrowth of the ectodermic collar begins first in the 6-mm. larvae dorsal to the solid endodermic oral collar and later the ventral portion of the collar is formed in the same manner. Preceding the formation of the ventral portion of the collar, there is mesenchyme between the ventral surface of the endodermal oral column and the ectoderm of the mandible, which is of ectodermal origin. This statement is made in spite of the fact that occasionally an endodermic cell is found here. Many of these can easily be shown to be walls of blood-vessels or isolated blood-cells which retain their endodermic characters, while others can be shown to be solid extensions of blood-vessels, and it is probable that all of them are concerned with blood-vessel

or lymphatic-vessel formation. However, when the ectodermal collar forms, its distinctness from the mesenchyme is quite evident, since the mesenchyme is loose in structure, while the ectodermal collar is dense and continuous with the deeper layer of the surface ectoderm. As to the later differentiation of the ectodermic collar, we find ourselves in agreement with Johnston ('10), who states that it gives rise to the dental ridges and teeth, while the endodermal lining of the oral cavity gives rise to the taste buds and oral epithelium.

Concerning the fate of the remaining cells of the ectodermic collar, we cannot make such definite statements. Aside from the teeth, the ectoderm gives rise to the dense tissue in which the teeth are imbedded. The dental ridges and teeth, however, disarrange the cells of the ectodermic collar where it comes into contact with mesenchyme, and the collar may possibly contribute to the mesenchyme, but the indications are against such an interpretation. We have followed the ectodermic collar up to the time when membrane bone begins to form around Meckel's cartilage and about the bases of the teeth. At this late stage one is forced to the uncertain method of tracing derivation by position mainly. Based on this criterion, the bone that forms about Meckel's cartilage comes from the mesenchyme of both ectodermal and endodermal origin, while the membrane surrounding the teeth seems to come from ectoderm only. It is certainly formed in the same dense tissue of the collar from which the teeth arise, although we cannot exclude the possibility of the migration of mesenchyme cells of mixed origin into the ectodermal collar. The late stage at which true membrane bone forms renders it unsafe to make definite statements concerning its derivation from ectodermal mesenchyme as distinct from endodermal mesenchyme because of the fact that both types are similar in structure, owing to the loss of yolk granules by the endoderm and the reduction in size of endodermal mesenchyme cells.

My conclusion, therefore, concerning the contribution of the lateral ectoderm to mesenchyme is that the lateral ectoderm, aside from the oral ectodermic collar and its derivations, is not

concerned in the formation of mesenchyme and that any delamination of cells from the lateral ectoderm is readily explained as concerned in the formation of other well-known structures. Dorsal and ventral to the oral cavity ectoderm forms the dense connective tissue in which the teeth are imbedded, but I find no evidence for the derivation of loose mesenchyme from the lateral ingrowth of ectoderm.

THE DIFFERENTIATION OF THE MESODERM AND NEURAL CREST IN THE HEAD

The possibility of following the differentiation of mesoderm into muscles, cartilages, and entodermal mesenchyme, and of neural crest into ganglia, ectodermal mesenchyme, and cartilages depends, as indicated earlier in this paper, upon easily recognized histological differences in ectodermal and entodermal cells. Ectodermal cells are smaller than entodermal cells, are usually pigmented, containing brown pigmented granules, and usually contain few yolk granules, or, if loaded with yolk granules, the granules are small and fairly uniform in size. Entodermal cells, on the other hand, are large and contain large numbers of large granules of varying sizes, and are not pigmented. When an embryo is stained with Delafield's haematoxylin and orange G, the contrast between neural crest and mesoderm is very striking.

These differences between ectodermal and entodermal derivatives can be followed in the urodeles up to the stage where the neural-crest ganglia, branchial muscles and eye muscles, branchial and cranial cartilages can be readily identified. One can follow the differentiation of the ectoderm and entoderm into mesenchyme with ease where the two types do not overlap. In areas of overlap even, the two types can be followed easily in the earlier stages when they lie in continuous sheets of cells. In the loose mesenchyme where the ectodermal and endodermal mesenchyme cells are mingled one can for a long time identify with certainty ectodermal and endodermal mesenchyme cells. The loose mesenchyme finally becomes homogeneous, entodermal cells assuming the appearance of ectodermal cells, and after this stage it is impossible to say which type of cell predominates.

It is a rather striking fact in the distribution of ectoderm and endoderm in the head that, in general, ectoderm gives rise to mesenchyme and cartilages located ventrally, that is, farthest from its source of origin, while mesoderm gives rise to mesenchyme in the dorsal regions of the head, which makes its location farthest from its source of origin. The loose entodermal mesenchyme of the head is derived in part from the mesoderm, which also gives rise to branchial muscles ventrally and to eye muscles dorsally. The actual formation of mesenchyme in these locations consists in the detachment of individual cells from the mesial surface of the more anterior and ventral muscle primordia, such as the masseter and temporalis, as well as from the more dorsal mesoderm from which eye muscles arise.

That portion of mesoderm which migrates beyond the muscle-forming area breaks down completely into loose mesenchyme. The mesoderm of the head, therefore, shows two types of tissue, a) dense masses of cells which can be followed into definitive branchial and eye muscles, retaining all the time their entodermal character up to the time muscle fibrils appear in the case of branchial muscles, and, b) loose mesenchyme which gradually loses its entodermal character and assumes the form of ectodermal mesenchyme.

The formation of ectodermal mesenchyme and cartilages from neural crest is really easier to follow than the mesodermal derivatives, except in the dorsal head regions from which the ectodermal mesenchyme withdraws as a continuous sheet. The neural crest, in its ventral migration, forms a continuous sheet of cells readily distinguished from mesoderm and lying lateral to the mesoderm. This continuous sheet of neural-crest cells gives rise, in its dorsal region, to the neural-crest ganglia and in other dorsal regions disintegrates into mesenchyme, which disappears as a continuous sheet in certain regions as indicated in figures 9, 10 and 11.

That portion of the neural crest which migrates into the ventral head region, that is, dorsal and ventral to the oral region and into the branchial bars, presents a quite different history. This ventral portion of the neural crest does not disintegrate. It not

only maintains its continuity, but grows extensively, surrounding the branchial muscles and filling the areas between muscles and epithelial structures. In this rather dense and continuous sheet of neural-crest cells there can be identified the still more condensed areas where the cartilages, such as Meckel's and the branchial cartilages, will form. Coincident with the formation of procartilage, there is going on a disintegration of the remaining neural crest to form loose ectodermal mesenchyme in much the same way as loose entodermal mesenchyme is formed from the mesoderm in more dorsal regions. Consequently, in the ventral head and branchial regions where the mesoderm gives rise to the muscles, most of the loose mesenchyme is derived from neural crest and is therefore ectodermal, while in the dorsal head regions, from which neural crest has disappeared as a continuous sheet, it is chiefly entodermal (mesodermal) mesenchyme.

Our conclusions concerning the metamorphosis of neural-crest cells into cartilage (excepting Miss Platt's conception of the behavior of lateral ectoderm) are so closely in accord with those of Platt, Dohrn, and Brauer that it is not necessary to give a detailed discussion of the process. The branchial cartilages all differentiate out of the ventralmost extension of the neural crest. They all show uniformly a stage where the neural crest is in the form of a sheet of cells surrounding the corresponding mesodermic branchial muscle primordium. Since this sheet of neural-crest cells forms a syncytium, it is true mesenchyme, although somewhat more compact than entodermal mesenchyme. Throughout the whole ventral head region and branchial region the differentiation of this primitive mesenchyme shows two types of behavior: *a*) Where no cartilages form its cells become detached from the sheet of neural-crest cells and form loose mesenchyme surrounding muscles and cartilages. This is practically pure ectodermal mesenchyme. The few entodermal cells found may prove usually to be growing blood-vessels or blood-cells; *b*) where a cartilage forms the neural-crest cells first increase in number, then condense, passing through typical procartilage stages, and finally form true cartilage. We find, in agreement with Miss Platt, that the anterior portion of the trabecular

bars, the palatoquadrate and Meckel's cartilages, all the branchial cartilages except the second basibranchial are formed from ectodermal mesenchyme, while the posterior portion of the trabecular bars, the parachordals and basal plate of the chondrocranium along with the occipital arch arise from entodermal mesenchyme. The roof of the chondrocranium arises very late and is assumed to come from entodermal mesenchyme on the basis of the distribution as shown in figure 11, although at the stage when the lateral walls of the cranium appear the mesenchyme is homogeneous. I am unable to determine the composition of the brain membranes and their homologues, the choroid and sclerotic coats of the eye, on account of the lateness of their formation, which occurs after the mesenchyme from which they form has become homogeneous. The cartilages listed above as ectodermic or entodermic in origin show, in addition to the fact that their development from ectoderm and entoderm can be followed, quite definite ectodermic and entodermic characters after they have assumed their definitive forms and after their cells are surrounded by hyaline material so dense that cell division within the cartilage has practically ceased.

The formation of the second basibranchial from entoderm needs a word of explanation. The second basibranchial of Miss Platt is the urohyal of some authors. It is the most caudal of the median branchial cartilages and lies ventral to the other branchial cartilages, except at its anterior attached extremity. It develops from mesoderm along with the paired geniohyoid muscles which are attached to its extremity. The mesoderm from which these three structures develop is continuous at first with the lateral borders of the alimentary canal from which other muscle primordia form. It retains for a long time its entodermal type of cell, so that there can be no question as to its origin. No explanation is offered of this curious fact further than that suggested by its position, which, as shown by a reference to figure 11, is at a point where ectoderm does not extend, that is, on the midventral line ventral to third, fourth, and fifth branchial bars.

The same explanation is offered for the double composition of the trabeculae. The anterior portions of the trabeculae form in a region dominated by ectodermal mesenchyme. This region lies between the two dorsal spurs just over the eye in figure 11, while the caudal portions of the trabeculae and the parachordals form in a region containing axial entodermal mesenchyme.

SUMMARY

1. The neural crest in the urodeles is incorporated in the neural canal at first and later erupted and then grows ventrally as a continuous sheet of cells. This ventral migration carries it into the ventral region of the anterior portion of the head and into the ventral portions of the mandibular and branchial bars.

2. The dorsal portion of the neural-crest sheet gives rise to the general cutaneous and general visceral portions of ganglia V, VII, IX, and X cranial nerves. The dorsal portion of the neural crest not concerned in the formation of ganglia disintegrates to form mesenchyme, which becomes mingled with entodermal mesenchyme in the dorsal head region.

3. The ventral portion of the continuous neural-crest sheet, after migrating into the ventral head region and into the mandibular and branchial bars, surrounds the primordia of branchial muscles growing from their lateral surfaces around to their mesial surfaces. This ventral portion of the neural crest later differentiates into loose ectodermal mesenchyme and into dense cartilage primordia, which pass through procartilage into typical cartilages.

4. The greater portion of the mesenchyme in the ventral head region and in the branchial regions is consequently ectodermal mesenchyme.

5. The cartilages arising from ectoderm are the anterior portion of the trabeculae, Meckel's cartilage, the palatoquadrate bar, and all the branchial cartilages except the second basibranchial or urohyal.

6. The mesoderm in the head gives rise to the branchial muscles, to the eye muscles, and to loose entodermal mesen-

chyme, which retains for a long time its entodermal characteristics. Entodermal mesenchyme later differentiates into cartilages forming the posterior portion of the trabeculae, the parachordals and base of the cranium, the occipital arch, the auditory capsule, and probably the lateral walls of the cranium.

7. The neural crest and mesoderm, where not concerned in the formation of ganglia and muscles, respectively, shift their positions extensively, but the net result of their migrations is to leave entodermal mesenchyme in the dorsal head regions, ectodermal mesenchyme in the ventral head regions, and mixed mesenchyme in the median longitudinal axis of the head. Where this overlapping occurs ectodermal mesenchyme is lateral in position, while entodermal mesenchyme is mesial in position. The loose axial mesenchyme lying in the median line between the brain floor and the dorsal surface of the mouth and pharynx is almost pure entodermal mesenchyme.

8. The lateral ectoderm, aside from the oral region, is not concerned in the formation of mesenchyme, but does give rise to the profundus ganglion, to lateral-line ganglia, to lateral-line organs, and to epibranchial ganglia.

9. The lateral ectoderm in the oral region gives rise to an ectodermic collar, which surrounds the solid entoderm of the oral region, and in this collar arise dental ridges and teeth and dense connective tissue in which the teeth are imbedded. We can find no evidence for the formation of loose mesenchyme from this ectodermic collar.

10. The histological grounds on which the distinctions in the behavior of ectoderm and entoderm rest are as follows: entoderm cells are larger than ectoderm cells: entoderm cells are rarely pigmented, while ectoderm cells usually are. Ectoderm contains small, fairly uniform-size yolk granules and loses them early, while entoderm contains large granules of irregular size and retains them much longer. In addition to these histological differences, the continuity of sheets of ectoderm and of entoderm cells is a valuable aid in following their distribution. The distinctions mentioned above can be observed after definitive ganglia, muscles, cartilages, and mesenchyme are formed.

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PLATE 1

EXPLANATION OF FIGURES

Figures 1, 2, 3, 4, 5 and 6 are transverse sections of *Plethodon glutinosus* at the level of the VII ganglion. The figures were drawn at a magnification of 275 and reduced one-half in reproduction. Figures 1, 2, 3, 4 and 5 are from larvae between 2 and 3 mm. in length. Figure 6 is from larvae 3 mm. in length.

- 1 Open neural canal stage.
- 2 Closed neural canal stage with the neural crest included in the neural tube.
- 3 Shows the 'eruption' of the neural crest. The neural crest forms part of the neural tube, but is easily distinguished from the tube.
- 4 The neural crest is largely outside the neural tube, but still forms a plug in its dorsal border.
- 5 Shows the neural crest almost completely outside the neural tube.
- 6 Shows the neural crest present in the form of typical cerebral ganglia.

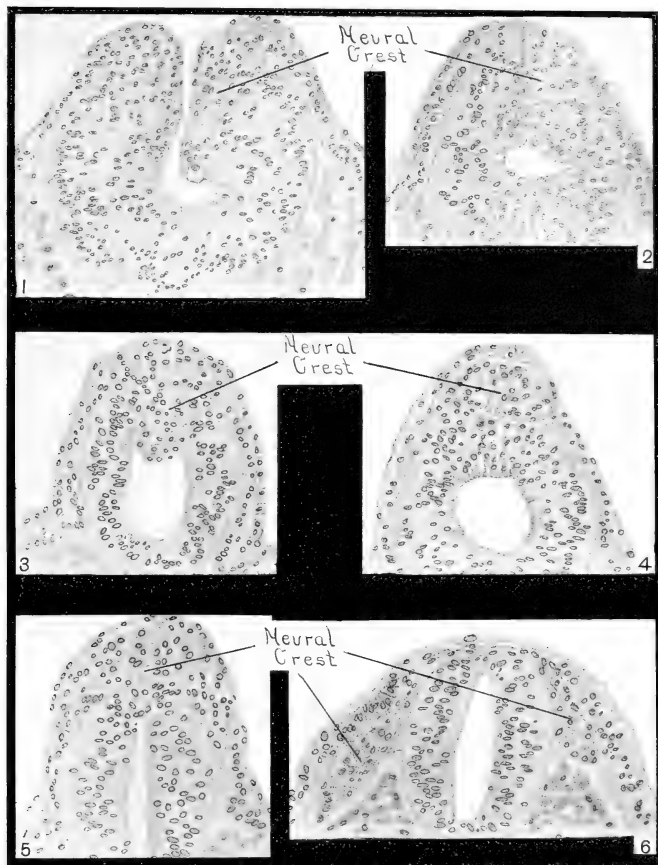


PLATE 2

EXPLANATION OF FIGURES

Figures 7 and 8 are flat reconstructions of the neural crest and mesoderm made by projecting the camera figures on coordinate paper. The plates give an accurate picture of the extent of the neural crest (diagonal lines) and mesoderm (stipple). The neural canal, notochord, alimentary canal, and sense organs are represented by lines. Both figures are from larvae 3 mm. in length, but the larva from which figure 8 is taken is six hours older than the larva from which figure 7 is taken.

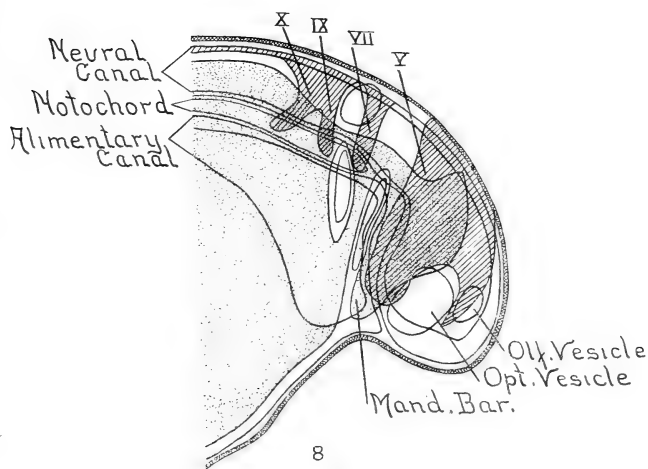
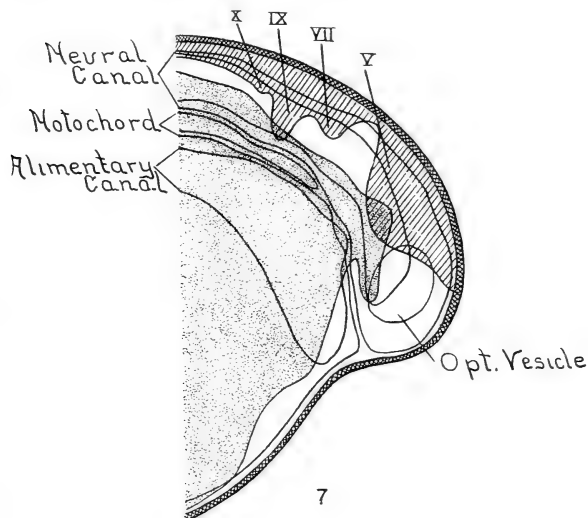


PLATE 3

EXPLANATION OF FIGURES

Figures 9 and 10 are reconstructions similar to figures 7 and 8. Figure 9 is from a larva 4 mm. long and figure 10 from a larva $4\frac{1}{2}$ mm. long. The neural crest is in diagonal lines and mesoderm in stipple.

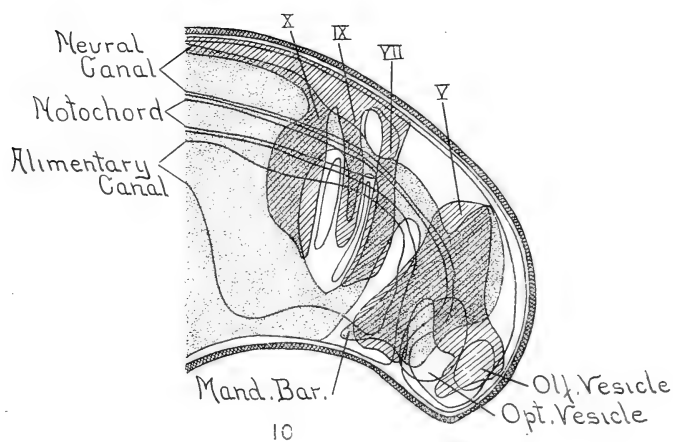
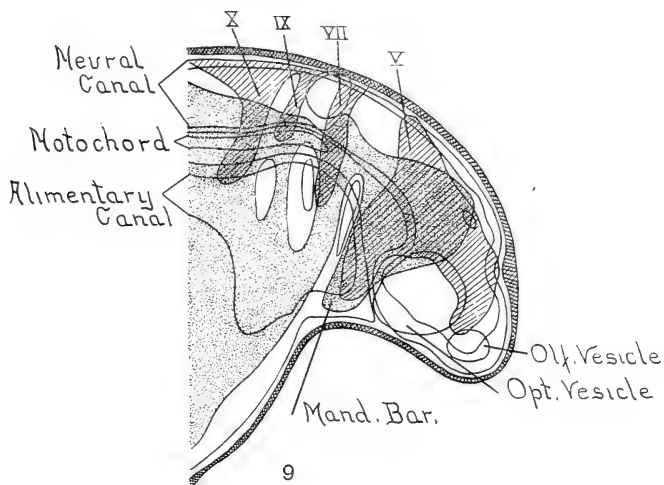
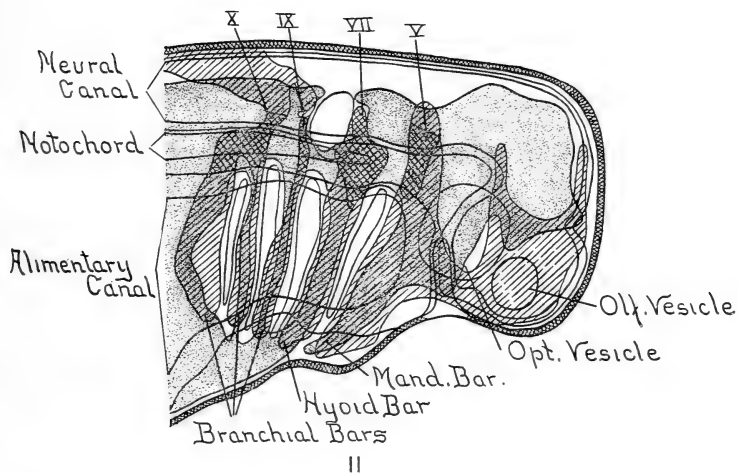


PLATE 4

EXPLANATION OF FIGURE

Figure 11 is a reconstruction similar to figures 7, 8, 9, and 10. This larva was 6 mm. long. Cerebral ganglia are represented by cross-hatched lines; neural crest by diagonal lines, and mesoderm by stipple. The neural canal, notochord, alimentary canal, and sense organs are represented by lines. The gill slits are unshaded. All of the neural crest below the ventral border of the cerebral ganglia will be converted into mesenchyme or cartilages.



Resumen por los autores, M. H. Cook y H. V. Neal,
Tufts College, Massachusetts

¿Son de origen endodérmico los botones gustativos de los
Elasmobranquios?

El presente trabajo representa un intento de determinación del origen ectodérmico o endodérmico de los botones gustativos de *Squalus acanthias*. Los autores han llevado a cabo un estudio de cortes de embriones de 7 a 80 mm., suplementado con disecciones de los estados jóvenes y adultos. En esta especie los botones gustativos están limitados a la región de la farínge, que en todos los estados de la ontogénesis está tapizada por endodermo. No se puede observar un avance marcado del ectodermo, ni siquiera en la región bucal. En el piso y techo de la farínge se forman escamas semejantes a las que caracterizan a la piel en los estados avanzados de la ontogénesis. De este modo parecen originarse en *Squalus acanthias* dos clases de órganos que generalmente se consideran como ectodérmicos, esto es, los botones gustativos y las escamas placoides.

Afirmar que los botones gustativos faríngeos y las escamas de *Squalus* son de origen ectodérmico implica la admisión de que el revestimiento endodérmico de la farínge desaparece por completo durante la ontogenia y es reemplazado por el ectodermo. No existe prueba alguna de tal sustitución. Estos resultados extienden a los Elasmobranquios la conclusión de Johnston ('98, '05, '10) de que los botones gustativos de los Teleósteos y Anfibios derivan del endodermo. También suman a las estructuras derivadas del endodermo las escamas faríngeas, que hasta el presente han sido consideradas como ectodérmicas, y así añaden otra excepción a la ley de la especificidad de las capas germinales.

Translation by José F. Nonidez
Cornell Medical College, New York

ARE THE TASTE-BUDS OF ELASMOBRANCHS ENDODERMAL IN ORIGIN?

MARGARET H. COOK AND H. V. NEAL

Tufts College, Massachusetts

FOUR PLATES (TWENTY-NINE FIGURES)

Scattered over the surface of the pharynx of the common spiny dogfish (*Squalus acanthias*) are numerous sense organs (taste-buds and placoid scales, figs. 28 and 29)—structures which morphologists generally have regarded as ectodermal. Moreover, the pharyngeal epidermis resembles that of the skin rather than epithelium such as that of the stomach and intestine, which is known to be of endodermal origin. Yet, as is perfectly well known, the pharynx of vertebrate embryos is primarily exclusively lined by endoderm. Are we to believe that the primary endodermal lining is secondarily replaced by ectoderm? That the stomodaeal ingrowth extends to the oesophagus? Or that the specificity of germ layers is not so precise as was formerly assumed? That endoderm may under certain conditions give rise to sense organs and placoid scales? Is it possible to settle these questions? The present paper is an attempt to do so on the basis of observations upon embryos of *Squalus acanthias*.

Taste-buds are known to be present in the pharynx of all vertebrates from cyclostomes to man. They were first described for fishes by Weber ('27) in the carp, and after him for other fishes by a number of observers. All of these correctly inferred their function. Some distinguished between pharyngeal taste-buds and organs of similar structure situated on the external surface of the body called 'end-buds' or 'terminal-buds.'

In many fishes and some amphibia terminal-buds occur over the whole outer surface of the body, extending even to the tail. This condition Herrick ('04), who demonstrated a similar function for terminal-buds and taste-buds, correlates with sluggish habits

and poor vision. In *Squalus acanthias*, a very active form, taste-buds are limited to the mouth and pharynx. Here they arise in the epithelial lining of the floor, the roof and the sides, including the visceral arches. They are first visible when the embryo is about 40 mm. in length. They are numerous and extend forward on the roof and floor of the pharynx to the region of the upper and lower jaws and posteriorly to the papillae of the oesophagus. Their distribution shows clearly in a 'pup' stage such as is represented in figure 28 of this paper.

The structure of taste-buds in *Squalus acanthias* is similar to that described for other vertebrates. They occur upon small papillae projecting slightly above the surrounding epithelium. The papillae are covered with a many-layered epithelium similar to that which covers the surface of the pharynx. At the apex of the papilla, however, the epithelial cells are modified into long, slender 'sense-cells,' each of which terminates externally in a short hair-like projection. Nerve fibers may be traced to the bases of such cells. Figures 1, 2, and 3 of this paper show three stages in the differentiation of taste papillae and 'buds.'

Bateson ('90) and Nagel ('94) were the first to attempt to demonstrate the function of these structures by experiment. They worked on a number of forms, including two dogfishes, *Scyllium canicula* and *S. catulus*. Bateson describes the distribution of taste-buds in the dogfishes as limited to the pharynx, although he adds that he would not presume to say that they may not be found also on the outer surface of the body. He failed to demonstrate that they play any important part in food-getting. This process, according to his observations, is controlled by the olfactory organ. Nagel, using dilute solutions of sour, bitter, and salty substances for taste, finds that the sense of taste is but feebly developed in *Scyllium*. He concludes that a sense of taste, such as in most animals is located in the mouth, is absent from the outer skin of all fishes.

Recent work by Sheldon ('09) on the smooth dogfish, *Mustelus canis*, which resembles *S. acanthias* in having the taste-buds limited to the pharynx, shows that these organs are concerned in reactions to the stimulus of bitter substances. To quinine

this form reacts in the mouth and spiracle only, that is, in the region bearing taste-buds, as was discovered by Parker ('08) for the catfish. But the whole surface of the body is sensitive to the stimulus of sour, salty, and bitter substances. By operations Sheldon was able to determine that these reactions of the general body surface are due to stimulation, not of the gustatory nerves which supply the taste-buds, but of the nerves of general sensation. These same nerves of general sensation were shown to give rise when stimulated to responses even in the mouth, where also taste-buds and their nerves take part in reactions. From these results Sheldon concludes, and in this he agrees with Herrick ('08), that in the dogfish the more specialized senses of smell and taste (as in the taste-buds) are derived from the primitive general chemical sense. Parker ('12), on the other hand, considers the olfactory sense the more primitive and derives from it the chemical sense which later gives rise to the taste-buds.

Just how much the gustatory nerves are stimulated through the secretory action of the so-called sense-cells no one has ever been able to determine. It has, however, been suggested by Botezat ('10) and Parker ('12) that these modified epithelial cells to which the name taste-buds is given may be primarily secretory, and that the nerves receive their stimulation through the response (secretion) of these cells to the stimulating substances. Materials, then, like quinine, would be tasted by the dogfish, because the cells of the taste-buds react to the quinine and secrete a substance which in turn stimulates the associated nerve. Such an explanation would rule out the term 'sense-cell' as applied to the groups of slender cells making up the taste-buds.

Turning, now, to the problem of the endodermic origin of the taste-buds in vertebrates, we find that Johnston ('05-'10) has been the chief proponent of the view that they are endodermal, and it is he who has given the most positive and convincing evidence in support of this view. Johnston ('05) finds that in *Petromyzon* and two bony fishes the taste-buds develop first in the endodermal lining of the pharynx and make their appearance on the outer surface of the body later at the time of hatch-

ing. In *Catostomus* and *Coregonus* taste-buds arise in the endodermal lining of the pharynx and are distributed over its surface and as far posteriorly as the opening of the air bladder. Johnston ('09, '10) finds even more positive evidence of the endodermal origin of the taste-buds in *Amblystoma*, in the embryos of which the pharynx is lined with endoderm 'to the very lips.' Taste-buds make their appearance within this endodermal-lined pharynx several days before the mouth breaks through. More direct evidence of the endodermal origin of the taste-buds could hardly be expected. Yet in this connection and in direct conflict with Johnston's assertion that an ectodermal stomodaeum is wanting in *Amblystoma*, we have the evidence given by Kingsley and Thyng ('04) of a stomodaeal ingrowth in *Amblystoma* and of a considerable ectodermal invagination even before the mouth breaks through. While this evidence by no means disproves Johnston's main contention that the pharyngeal taste-buds are endodermal in origin, it is sufficient to make doubtful the exact amount of ectodermal ingrowth and to this extent to open up the possibility of an ectodermal origin of the taste-buds. Landacre ('07) supports Johnston's view of the endodermal origin of the taste-buds on the basis of observations upon *Ameiurus* embryos. He finds, however, that the 'terminal-buds' of the skin, which are so similar to the taste-buds, are ectodermal in origin. Keibel ('12) holds that in man the taste-buds are "probably derived from the entoblast. The majority of the taste-buds lie undoubtedly within the entoblastic territory, and even although epithelial encroachments are possible, yet it seems difficult to suppose that the ectoblast has penetrated into the region of the larynx."

The opinion that taste-buds are endodermal in origin seems strengthened by the evidence presented in sections of *Squalus* embryos. An examination of sections of the pharynx of this form shows that the whole pharyngeal cavity is endodermal in its origin and that there is little or no inward migration of the ectoderm into the pharynx except in the region of the upper and lower jaws, the epidermis of which is ectodermal. Until the time of perforation of the mouth and visceral clefts it is possible

in this form to distinguish ectoderm and entoderm by differences in staining properties and the presence of large yolk granules in the endodermal cells. After this rupture the limits of the two are more difficult to determine. Evidence, however, of the degeneration of the endodermal lining of the pharynx or of active extension of the ectoderm is wholly lacking. The hypophysis soon loses its primary connection with the ectoderm from which it arises and from it therefore evidence of ectodermal ingrowth into the mouth is not obtainable as in Amniote embryos. As the embryo continues to grow the upper lip region is pushed backward slightly and the ectoderm is carried with it forming the dental ridge, as is shown in figure 16.

Throughout the formation of the pharynx and of pharyngeal structures, such as the thyroid and gill pouches, it is the endoderm which is the active layer. And it is within the endoderm that the taste-buds first make their appearance in a 45-mm. embryo by the local thickening of the epidermis and the differentiation of cells of the stratum germinativum. To assert that the taste-buds in the pharynx of *Squalus* are ectodermal would necessitate the assumption—for which there is not a particle of direct evidence—that the primary endodermal lining of the pharynx is completely supplanted by ectodermal ingrowth.

In connection with the study of the histogenesis of taste-buds in *Squalus* another problem presents itself in the appearance in late embryonic stages of pharyngeal placoid scales, the distribution of which, as is shown in figure 29, is somewhat more restricted than that of the taste-buds. There are relatively few scales upon the roof of the pharynx, while they are abundant upon its floor, where in the region of the basibranchial cartilages they tend to conceal the taste-buds. The number of scales in the pharynx varies considerably in different individuals. In one individual examined there were so few that they could be detected only by boiling the epithelium of the roof of the pharynx in strong KOH solution. Yet in some individuals they may be easily distinguished with a hand lens or by rubbing the fingers anteriorly over the surface of the pharynx, since the scales like the pharyngeal teeth of teleosts are directed backward.

Pharyngeal scales in *Squalus* are similar to, although not identical in structure with, the placoid scales of the outer skin. They differ from them, however, in their origin. The placoid scales of the outer skin and the teeth of the upper and lower jaws derive their enamel layer from the embryonic ectoderm. Pharyngeal scales, however, form their enamel layer from the endoderm. The reasons for this conclusion are identical with those which convince us of the endodermal origin of the taste-buds. The pharyngeal scales, however, arise later than the taste-buds. No developed pharyngeal scales appear in the pharynx of a 'pup' stage in which the taste-buds are well developed and numerous (fig. 28). Sections of such a stage, however, show the anlagen of the pharyngeal scales (figs. 4 and 9). In such a section as that shown in figure 4 it may be seen that the same layer of the epidermis gives rise to the sense-cells of the taste-bud and to the enamel layer of the pharyngeal scale. Entoderm, therefore, it would appear, may give rise not only to sense organs, but to scales such as usually are conceived as ectodermal, that is to say cutaneous, in origin.

Against such a conclusion two chief objections may be urged: first, that it is highly improbable, since it conflicts with the principle of the specificity of the germ layers, and, second, that the acceptance by morphologists of the annelid ancestry of chordates justifies the assumption of an ectoderm-lined pharynx in the latter comparable with the stomodaeum of annelids. It is very doubtful, however, that such considerations will seem to many morphologists to outweigh the direct evidence from ontogenesis presented by Johnston and Landacre and in the present paper. It is hardly necessary to suggest that a biological principle is neither a self-evident truth nor a universal law, but a generalization or hypothesis usually formulated before a complete knowledge of the evidence. In the phenomena of budding in colonial tunicates and in the regeneration processes of chordates may be found exceptions to the principle of the specificity of germ layers. That endoderm may produce taste-buds and placoid scales is surely not more surprising than that muscle may be regenerated from ectoderm or the lens of the eye from mesen-

chyma, as has been demonstrated (Morgan, '01). In our conclusions concerning organic processes we have to reckon with the fact of organic plasticity as well as with that of specificity of tissue.

In the light of the considerable doubt that attaches to the annelid hypothesis of chordate ancestry, it is perhaps sufficient simply to suggest that such an hypothesis forms a most insecure foundation for deductions concerning the derivation of the lining of the chordate pharynx. The annelid hypothesis certainly seems less convincing than it did formerly. Yet it seems as if in our thinking we had tacitly assumed on the basis of that hypothesis a considerable ectodermal invagination into the pharynx. Such a prejudice is not supported by the ontogenetic evidence.

A brief summary of the arguments which have been presented for and against the endodermal origin of taste-buds and pharyngeal scales may now be given. In favor of the ectodermal derivation of these structures it may be urged that:

1. The resemblance of the histological structure of the pharyngeal epidermis to that of the mouth and skin—which are known to be of ectodermal origin—suggests a similar derivation for both. Furthermore, taste-buds and pharyngeal scales in *Squalus* structurally resemble cutaneous organs of known ectodermal origin. Such an argument, however, leads logically to the conclusion that the mucous lining of the esophagus is also ectodermal—a conclusion contradicted by the ontogenetic evidence. Are we to conclude, also, that because the epidermis of the gills of fishes does not resemble that of the pharynx and skin it is therefore not ectodermal in origin?

2. Taste-buds occur in the skin of some fishes in regions where their ectodermal origin seems indisputable (Herrick). It may be urged that it is highly improbable that the same sort of structure should arise independently from different germ layers. This argument seems strengthened by the fact that associated with the taste-buds in *Squalus* are placoid scales structurally comparable with those derived from the outer skin. Is it possible to believe that the specificity of the germ layers is so slight that identical structures may develop from both endoderm and ectoderm?

3. Since all other nervous receptor cells are ectodermal in origin, the presumption is wholly in favor of the ectodermal origin of the hair-cells of the taste-buds. This deduction, however, is based upon the assumption that the hair-cells of the pharyngeal taste-buds are nervous receptors. If they are glandular, as Botezat ('10) and Parker ('12) have assumed, the deduction is a logical non-sequitur.

4. That an ectodermal stomodaeal invagination occurs in vertebrate embryos is an established fact. The persistence in some animals of the connection of the ectodermal hypophysis with the roof of the embryonic mouth makes it probable that in these forms the ectodermal ingrowth extends as far posteriorly as the eustachian tubes. A relatively slight continuation of this process of ingrowth would line the pharynx with ectoderm. In the light of this positive evidence of extensive invagination of the ectoderm, disagreement with the opinion of Keibel ('12, p. 183), that "it is difficult to suppose that the ectoblast has penetrated into the region of the larynx," might seem not unreasonable.

5. The divergence in the conclusions of Kingsley and Thyng ('05) and Johnston ('10) regarding the amount of the ectodermal invagination in *Amblystoma* might appear to justify some doubt as to the certainty of the conclusion reached by the latter. Johnston seems not to have taken into consideration the facts presented in the former paper.

The following considerations, however, favor the conclusion that the pharyngeal lining with its associated taste-buds and placoid scales are derived from the entoderm:

1. The similarity of structure of the pharyngeal lining and the skin by no means proves a similar genesis or derivation. The similarity may be the result of convergence. Many instances of the convergence of organic structures from dissimilar beginnings are known. The force of the doctrine of the specificity of the germ layers has been greatly weakened by abundant evidence of the plasticity of regenerating tissue.

2. The pharynx of all vertebrate embryos is lined primarily with endoderm (figs. 5 to 27 of this paper). Direct evidence

that by a process of substitution this endodermal lining is secondarily replaced by ectoderm has never been given. The demonstration of an ectodermal dental ridge cannot be considered to be a demonstration of an ectodermal lining of the pharynx. It is equally fallacious to conclude that because annelids have an ectodermal foregut vertebrates must have an ectodermal pharynx. On the contrary, direct observation proves that the lining of the pharynx of vertebrate embryos is endodermal in origin.

3. "The presumption, from the standpoint of nerve distribution, is all in favor of the origin of taste-buds from endoderm" (Johnston, '10, pp. 64, 65), since they are "innervated by the facial nerve which is strictly a nerve related to endodermal surfaces in all vertebrates except those fishes in which the taste-buds spread into the outer skin."

CONCLUSIONS

1. Taste-buds in *Squalus acanthias* are limited to the pharynx, where they are distributed over the floor, the roof, and the gill pouches.

2. The structure of the taste-buds resembles that of taste-buds in other forms. They are groups of slender cells slightly raised above the surface into a papilla. Each cell bears, externally, a hair-like process, and is connected internally with a nerve ending.

3. Taste-buds in *S. acanthias* are derived from the endoderm. They develop from the epithelial lining of the pharynx which at all stages shows itself as endodermal, there being no indication at any period of development of a migration inward of the ectoderm, except the slight invagination which forms the dental ridge.

4. The pharyngeal scales arise in late embryonic stages. They resemble placoid scales in structure, but are derived from the endodermal lining of the pharynx.

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PLATES

ABBREVIATIONS

Arc.vsc.1-6, visceral arches 1-6
ar'ent., archenteron
brs.vsc.1-6, visceral pouches 1-6
cd., chorda dorsalis
cl.n'bl., neuroblastic cell
d.r., dental ridge
ec'drm., ectoderm
en'drm., entoderm
fil.br., branchial filaments
fis.vsc.1-6, visceral clefts 1-6
gl.th., thyroid gland
hyp., hypophysis
i'fb., infundibulum

m., mouth
m.pl., mouth plate
mand., mandibular cartilage
n'v., nerve
olf., olfactory pit
ph., pharynx
pl.sc., placoid scale
sp., spiracle
st.germ., stratum germinativum
t.b., taste-bud
t.pap., taste papilla
tb.n., neural tube
vil.oes., oesophageal villi

PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 10 were drawn with oil-immersion objective and reduced a little over one-half in reproduction. They illustrate various stages in the development of taste-buds, pharyngeal scales, and the formation of mouth and gill-slits.

1 An early stage in the development of a taste-bud and papilla as seen in a section of a 60-mm. *Squalus* embryo. Differentiation begins as a process of elongation and aggregation of a group of cells in the stratum germinativum of the pharyngeal epidermis which is of endodermal origin.

2 A section of an older taste papilla taken from a 90-mm. *Squalus* embryo, showing a portion of the associated sensory nerve.

3 A section of a fully differentiated taste papilla and 'bud' as seen in the 'pup' stage of *Squalus*.

4 A section of the pharyngeal lining of a 'pup' stage, showing to the left the anlage of a pharyngeal scale and to the right a (somewhat damaged) taste papilla.

5 A portion of a median sagittal section of a *Squalus* embryo (Balfour's stage K), showing the relations between ectoderm and endoderm in the region of the degenerating buccal plate ('preoral lobe'). The presence of large yolk granules in the endodermal cells serves at this stage to distinguish them from the ectodermal cells of the hypophysial invagination (above).

6, 7, and 8 Successive stages in the formation of a visceral cleft, showing the ectodermal-endodermal relations in frontal sections of a *Squalus* embryo (stage K). It is the endoderm which persists longest in the region of perforation of the cleft. At this stage the ectoderm stains more intensely than the endoderm so that their limits are easily distinguishable. The endoderm is the more active layer of the two in the process of visceral cleft formation.

9 A developing scale as seen in a section of an older 'pup' stage through the pharyngeal epidermis.

10 An enlarged portion of the upper jaw of a 60-mm. *Squalus* embryo, showing the region of transition between ectoderm and endoderm. At neither this nor any other stage it is possible to discover evidence of degeneration of the endoderm or of the rapid proliferation of ectoderm which would manifest itself if the ectodermal ingrowth were to supplant the primary endodermal lining.

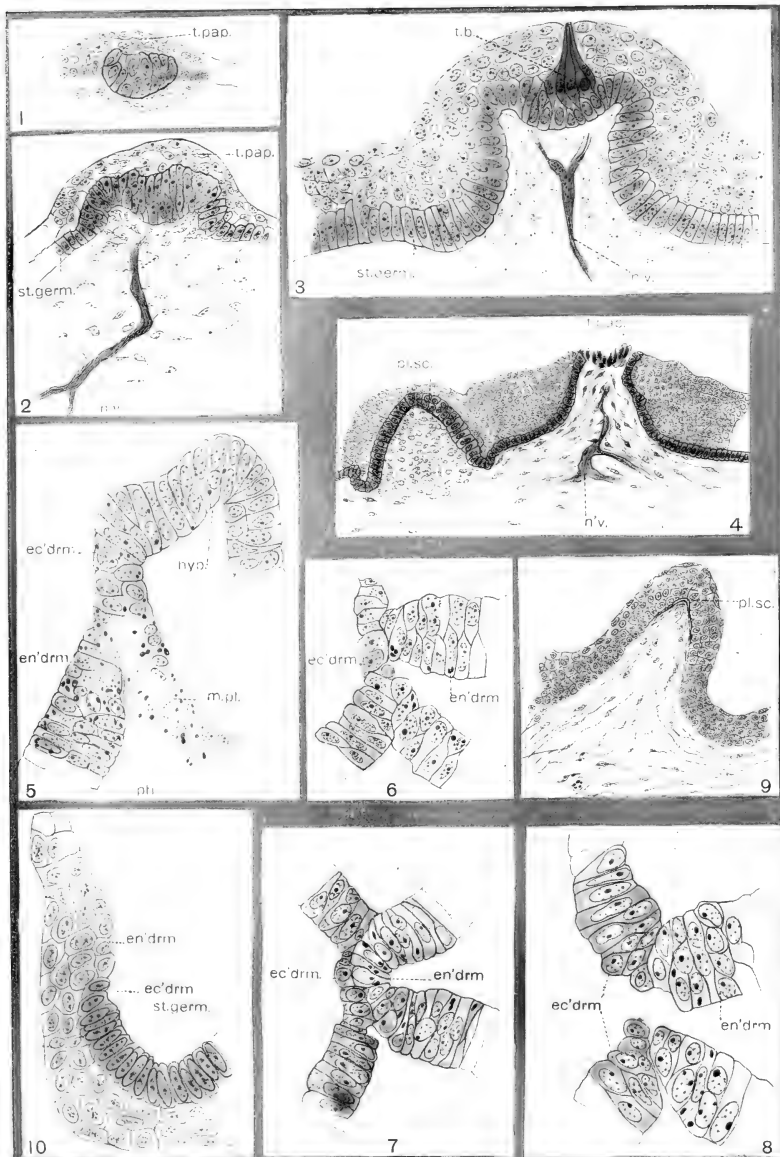


PLATE 2

EXPLANATION OF FIGURES

Figures 11 to 17 are drawn from median sagittal sections of *Squalus* embryos of different stages showing the limits and mutual relations of the ectoderm and endoderm in the region of the mouth and pharynx. The visceral pouches and clefts were superimposed from in toto preparations. Endodermal structures are cross-hatched and nervous system stippled in all drawings.

11 and 12 Median longitudinal sections of *Squalus* embryos in Balfour's stages G and H, showing the relations of the endoderm and ectoderm before the perforation of the buccal plate.

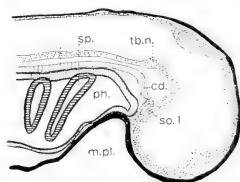
13 A median longitudinal section of stage I following the partial perforation of the buccal plate. The deeply staining properties of the ectodermal cells and the coarse yolk granules in the endodermal cells make easy the determination of the boundaries between ectoderm and endoderm at this stage. Two visceral clefts and two pouches formed.

14 In the region of the hypophyseal invagination a remnant of the double-layered buccal plate remains as the so-called preoral lobe. There are three visceral clefts and two pouches. Stage K.

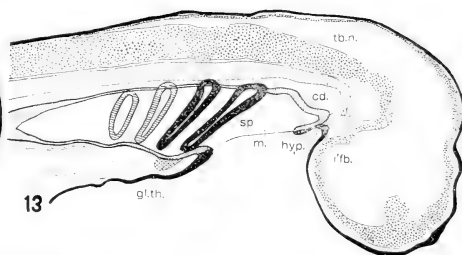
15 Stage L. The hypophysis still retains in connection with the ectodermal stomodaeum. The pharynx still retains its endodermal lining. The ectodermal ingrowth slight.

16 A median longitudinal section of a 60-mm. embryo in which taste papillae and buds are distinguishable in both floor and roof of the pharynx. The ectodermal ingrowth does not extend farther than the limits of the upper and lower jaws.

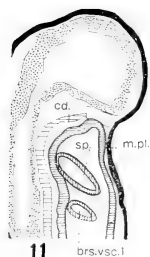
17 A more enlarged drawing to show the relations of ectoderm and endoderm in the region of upper and lower jaws. The taste-buds are limited to the epidermal surfaces formed from endoderm.



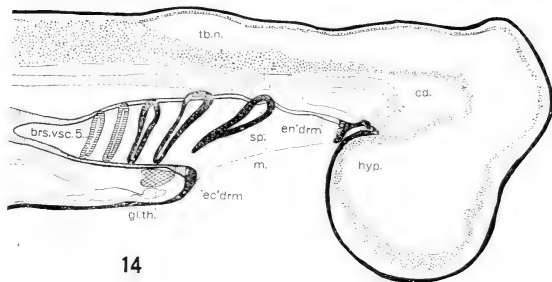
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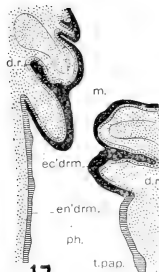
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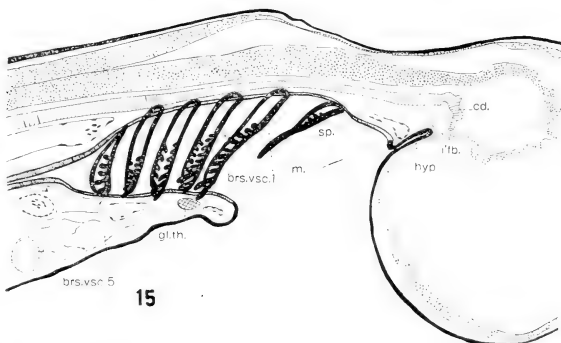
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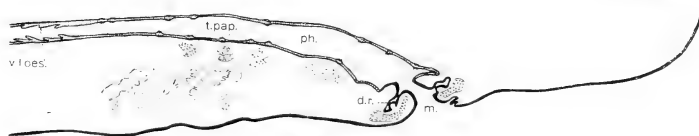
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PLATE 3

EXPLANATION OF FIGURES

Figures 18 to 27 are taken from cross-sections and frontal sections of *Squalus* embryos, stages G to L, to show the relations of the ectoderm and endoderm in the pharyngeal region. Subsequent to the perforation of the visceral clefts the limits of the ectoderm and endoderm are difficult to determine and the diagrams are to be understood as only approximately accurate.

18 A cross-section of a *Squalus* embryo (stage G) through the first (spiracular) pouch.

19 A cross-section through the same pouch (stage H). In the region of the future cleft the ectoderm thins out more rapidly than does the endoderm.

20 A cross-section (stage I) through the first and second pouches.

21 A cross-section (stage K) through the first and second pouches and the mouth which has broken through at this stage.

22 A frontal (horizontal) section of stage H, showing the endodermal out-pocketings to form the first, second, and third visceral pouches.

23 A frontal section of a *Squalus* embryo (stage I) cut dorsal to the hypophysis, showing the formation of the first four visceral pouches.

24 A frontal section of a *Squalus* embryo (stage I) at the level of the hypophysis, showing the formation of two visceral clefts and two pouches.

25 A frontal section of a *Squalus* embryo (stage K) dorsal to the level of the hypophysis, showing the ectodermal-endodermal relations in the region of the pharynx.

26 A frontal section of a *Squalus* embryo (stage L) cut at the level of the hypophysis, showing the extent of the pharyngeal epidermis.

27 A horizontal section of the same stage as figure 26, but cut at a higher level.

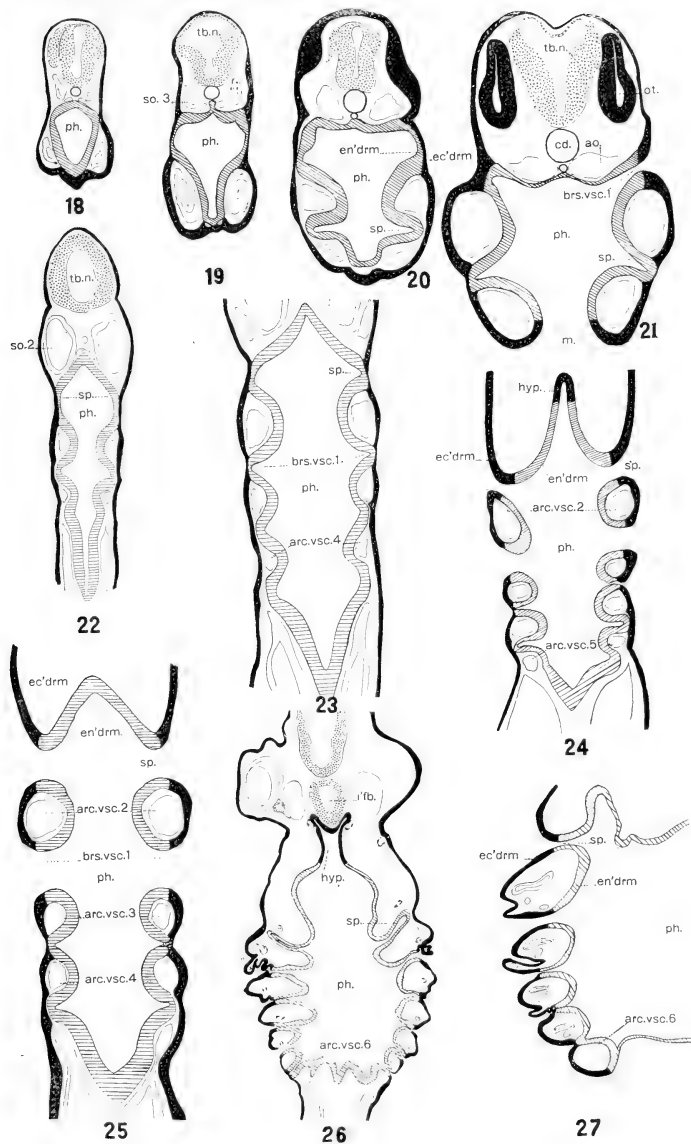


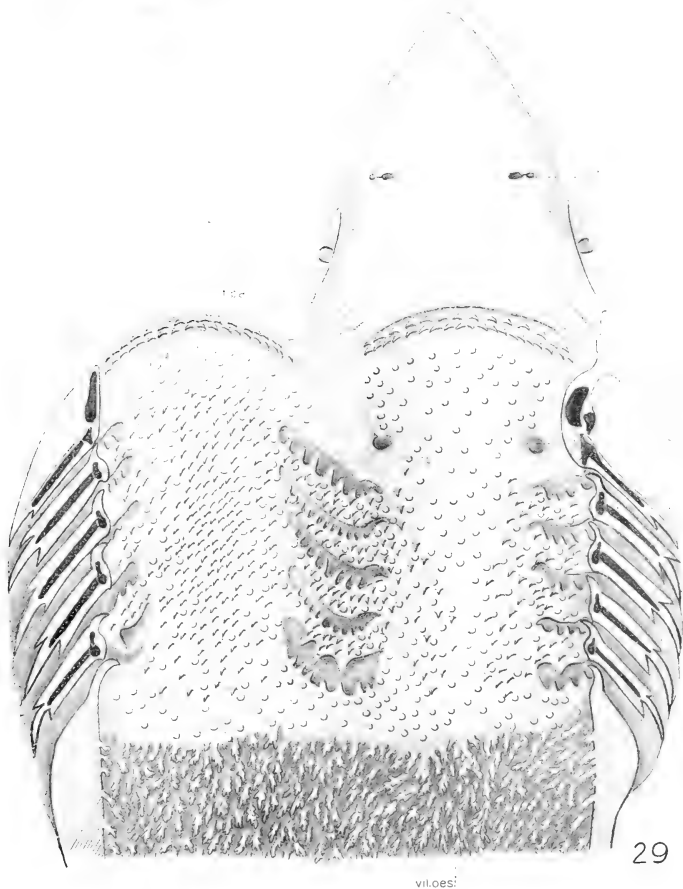
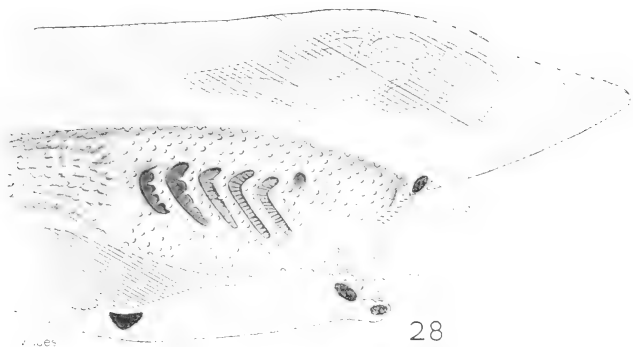
PLATE 4

EXPLANATION OF FIGURES

Figures 28 and 29 show the distribution of taste papillae and pharyngeal scales in the region of the mouth and pharynx of *Squalus acanthias*.

28 The left half of a dogfish, 'pup' stage, showing the distribution of taste papillae over the floor, roof, and sides of the pharynx.

29 The pharynx of an adult spiny dogfish laid open so as to show the distribution of taste papillae and pharyngeal scales. The floor of the pharynx is reflexed to the left in the figure.



Resumen por el autor, H. V. Neal,
Tufts College.

Nervios y plasmodesmos.

1. Antes del estado de 4.5 mm. no existen conexiones protoplásmicas entre el tubo neural y los miotomos en los embriones de *Squalus acanthias*. 2. La substancia neurofibrillar aparece en las primeras conexiones protoplásmicas entre el tubo neural y el miotomo. En estas conexiones primarias aparecen neurofibrillas intensamente teñidas que pueden seguirse hasta los neuroblastos bipolares situados en el tubo neural. La idea de que las conexiones protoplásmicas primarias están constituidas por plasmodesmos no diferenciados está basada en métodos neurológicos inadecuados. 3. En estados anteriores al establecimiento de conexiones entre el tubo neural y el miotomo, ciertas células medulares, en zonas en las cuales más tarde aparecen los esbozos de los nervios, presentan teñidas con el método de Bielehowsky-Paton un neuro-retículo intensamente coloreado. En estados un poco más avanzados, cuando se establece la conexión neuromuscular se encuentran células neuro-reticulares semejantes, unidas con neurofibrillas que se extienden en los esbozos de los nervios del modo característico de los neuroblastos medulares teñidos con los métodos neurofibrillares específicos. La evidencia sobre la presencia de una substancia neurofibrillar semejante en todas las partes del esbozo del nervio confirma la inferencia de que las conexiones neurofibrillares se establecen no mediante células indiferentes, sino por neuroblastos medulares, según mantienen los partidarios de la teoría de Bidder-Kupffer. Las células indiferentes solamente participan en la formación de los esbozos nerviosos en los estados más avanzados mediante un proceso de emigración desde el tubo neural.

Translation by José F. Nonidez
Cornell Medical College, New York

NERVE AND PLASMODESMA

H. V. NEAL

Tufts College, Massachusetts

FIVE FIGURES

That neuraxones develop as processes of ganglion cells scarcely admits of reasonable doubt in the light of the evidence now in our possession. Few today would challenge the truth of Harrison's ('13) assertion that "the work on the cultivation of tissues may be said without reserve to have completely proved the correctness of the conception of His and Ramón y Cajal." The long controversy between the adherents of the cell-process theory and the supporters of the cell-chain hypothesis of neurogenesis has been finally settled in favor of the former. To say this, however, is by no means to assert that all problems of neurogenesis have been solved. Such has never been the claim of Harrison or any other supporter of the Bidder-Kupffer hypothesis. There yet remain unsolved a number of controverted questions of great interest, some physiological and some morphological, which neither tissue cultures in vitro nor observations upon sectioned material have been able to solve. Familiar examples of such are the problems of the genesis of the nerve sheaths and of the sympathetic cells. Further questions are suggested by the phenomena of nerve regeneration (Boeke, '16).

One of the disputed points in the histogenesis of nerve is whether or not there exists previous to nervous (neurofibrillar) connection between neural tube and myotome a connection by means of undifferentiated protoplasmic threads, or plasmodesmata, or 'fasernetz' of Szily ('04). Among those who assume the existence of such plasmodesmata it is a matter of dispute whether the plasmodesmata are primary—the result of incomplete cell division, as first stated by Hensen ('64)—or secondary—the result of protoplasmic outflow of medullary cells as first

stated by Bidder and Kupffer ('57). Furthermore, among those who hold that neuromuscular connections are secondary there is disagreement as to whether such connections are effected by indifferent (glia or neurilemma) cells, as suggested by the Hertwigs ('78) and recently maintained by Held ('09), or by the outgrowth of the neuraxone processes of medullary neuroblasts, as believed by advocates of the Bidder-Kupffer hypothesis. Not until these questions are adequately answered is it likely that the investigation of neurogenesis will cease.

The invention in recent years of specific neurofibrillar stains and the demonstration of neurofibrillae in the earlier stages of neurogenesis have led to the general adoption of the aphorism of Apáthy that "there is no nerve without neurofibrillae" as a criterion of nervous structure. How greatly needed such a criterion has been is only too well known to those familiar with the literature of neurogenesis. In the absence of such a criterion of nervous structure, it has been hitherto possible to find in any cellular strand extending to or from the nervous system the evidence of 'primitive nerves' wherever they were demanded for schemes of ancestral metamerism. However, notwithstanding the application of improved methods of demonstrating neurofibrillae in embryonic nervous tissue, it has been found impossible up to the present to demonstrate the presence of neurofibrillae in the primary connections between nerve and muscle, that is to say, between neural tube and myotome. The existence of such neurofibrillae in the plasmodesmata of *Squalus* embryos is asserted in this paper for the first time in vertebrate embryos.¹

The present paper raises three controverted problems in nerve histogenesis:

1. Are the connections between nerve and muscle primary or secondary?
2. Are neuromuscular connections primarily undifferentiated plasmodesmata, as asserted by Paton ('07) and Held ('09), or are they primarily neurofibrillar?

¹ A preliminary report of the conclusions reached in this paper was made before the American Society of Zoologists at Philadelphia, December 29, 1914, and an abstract reprinted in *Science*, 1915, vol. 41, pp. 435-436. War work has delayed the appearance of the final paper.

3. Are neuromuscular connections effected by indifferent—neurilemma or glia or mesenchyma—cells or by medullary neuroblasts? All three questions have been discussed at length in papers by Paton ('07), Held ('09), and the writer ('14), in which the voluminous literature has been reviewed. The reason for raising again the three questions stated above is that, while none of the investigators up to the present has been able to demonstrate neurofibrillae in the primary protoplasmic connections between the neural tube and the myotome, the writer has been able to discover them in preparations made by the Bielschowsky-Paton process. The evidence is presented in the five figures of this paper. The divergence in the conclusions reached by Paton and Held on the one hand and by the writer on the other are essentially the result of the capriciousness of special neurofibrillar stains as applied to the earlier stages of nerve histogenesis. Not until after some years of experimentation with the Bielschowsky method and not until after the publication of the 1914 paper on the morphology of the eye-muscle nerves did the writer succeed in staining the neurofibrillae in the plasmodesmata. The present paper is therefore to be considered as a supplement to the one published in 1914.

Turning, now, to the first of the questions raised above, the writer can only reiterate the assertion made in earlier papers that in embryos of *Squalus acanthias* of stages previous to 4.5 mm. there is not the slightest evidence of protoplasmic connection between neural tube and myotome. Figures 1 and 2 of this paper reproduce faithfully the relations which obtain previous to the appearance of definitive nervous connection. These figures represent sections cut transversely through the middle of a myotome where in the later stages the anlagen of the motor nerves make their appearance. In some preparations it is possible to demonstrate in the plasma-filled space between neural tube and myotome a minimal amount of vacuolated coagulable material, invisible in most preparations made by the usual methods of fixation and staining, and lacking the staining properties of cell protoplasm. The 'Fasernetz' of Szily ('04) and the plasmodesmata of Paton ('07) and of Held ('09) make

their appearance later in the manner described below. Protoplasmic connection between tube and myotome in *Squalus* is secondary and not primary.

The failure of Paton, Held, and the writer to discover the presence of neurofibrillae in the first protoplasmic connections between neural tube and myotome led all three to assert that neurofibrillas are secondarily differentiated within non-nervous, undifferentiated 'plasmodesmata.' The divergent views as to the details of this process have been summarized by the writer ('14, pp. 35-41) and need not be repeated here. In that paper the writer asserted that "medullary cells by a process of outgrowth, form the first protoplasmic connection between tube and myotome" and that later within these processes the neurofibrillae make their appearance. Bielschowsky-Paton preparations also showed the presence of a neuroreticulum within bipolar neuroblasts lying in the somatic column of the spinal cord and in the zones opposite the middle of the somites where later the plasmodesmata make their appearance. Similar neuroblasts containing a deeply staining reticulum are shown in figures 1 and 2 of this paper. But at the time the 1914 paper was published no neurofibrillar structures were seen in the plasmodesmatous connections at the time of their first appearance. Since that time, however, they have been seen in a number of series prepared by the Bielschowsky-Paton method. Figures 3 to 5 of this paper show the presence of neurofibrillar substance within the 'plasmodesmata' of Paton and Held. The justification for calling the deeply staining material of the plasmodesmata 'neurofibrillar' consists in the fact that it stains precisely like the neurofibrillar network present within the neuroblasts which by their outgrowth form the plasmodesmata and in the further fact that it is possible to trace the neurofibrillar network of the neuroblastic cells from the tube into the plasmodesmata as is seen in the figures mentioned. Its granular appearance within the plasmodesmata may be interpreted as the result of cutting a fibrillar network transversely. In those instances where the neuraxone processes of the medullary neuroblasts are cut lengthwise, as in figures 3 and 4, the neurofibrillar substance appears

as heavy 'fibrils' as described by Paton. The affinity for neurofibrillar stains is especially marked at the lower, advancing extremity of the nerve anlage where Paton ('07) found the first indications of neurofibrillar substance in his preparations. From this evidence the conclusion seems warranted that the primary connections between neural tube and myotome are truly nervous, just as the experiments of Paton ('07) upon the reactions of elasmobranch embryos at the time the plasmodesmata are formed would lead us to infer.

The answer to the third question is already implied in the foregoing discussion. As has already been stated, in stages before protoplasmic connection between tube and myotome is effected certain medullary cells in zones where later nerve anlagen make their appearance show in Bielschowsky-Paton preparations a deeply staining neuroreticulum. Such cells show a pronounced polarity and tend to assume a spindle shape. The staining material of the reticulum is peripheral in position and extends throughout the entire length of the cell, showing a tendency to become more densely aggregated at the outer extremity of the cell. Not infrequently the end of the cell appears to extend along the inner surface of the external limiting membrane of the tube just as if the neuroblastic cell were in the process of elongation in the direction of its long axis and the movement of protoplasm were deflected in direction by the resistance offered by the limiting membrane of the medullary wall (fig. 1). The neuroreticulum is densely aggregated in the elongated peripheral portion of some cells so as to appear in such as a heavy deeply stained neurofibril.

In slightly later stages, when protoplasmic connection with the myotome has been established in the manner described in detail by the writer in earlier papers ('14), similar neuroblastic cells with deeply staining processes are seen to extend into the nerve anlagen (figs. 3 to 5), that is to say into the so-called plasmodesmata. That these plasmodesmata are primarily formed by the protoplasmic outflow of similar neuroblastic cells has already been asserted by the writer ('14. Note especially figs. 4 to 7, pls. 1 and 2). The plasmodesmata, in other

words, are not 'paths' utilized by the neuraxones as a means of conduction and material for growth, as assumed by Held ('09), but are *ab initio* nervous. The participation of indifferent (neurilemma or glia) cells in the formation of the motor nerve anlagen in elasmobranchs by means of the migration of medullary cells along the nerve anlagen ('plasmodesmata') has been described by many, including the writer ('03-'14), but it is not such cells which form the primary plasmodesmata. Admitting the possibility that in the plasmodesmatous connection shown in figures 3 to 5 of this paper processes of indifferent, non-neuroblastic cells occur, nevertheless the important fact remains that the primary neuromuscular connections were not formed by these indifferent, non-nervous elements. Their relations to the nerve anlagen are secondary and not primary.

In the light of the evidence that neuromuscular connections are *ab initio* nervous, there seems to be little justification for the application of the term 'plasmodesmata' to them. What we are actually dealing with in the case of such structures are nerve anlagen, or neuraxones and their sheaths. Why then term them plasmodesmata, thereby suggesting that they are merely non-nervous, undifferentiated intercellular connections?

SUMMARY

1. Previous to the stage of 4.5 mm. there are no protoplasmic connections between tube and myotomes in *Squalus* embryos.

2. Neurofibrillar substance is present in the primary connection between tube and myotome. Within the plasmodesmata of Paton ('07) and Held ('09) may be found in adequately stained Bielschowsky-Paton preparations deeply stained neurofibrils which may be traced to bipolar neuroblasts within the neural tube. The assertion that the primary connection between muscle and nerve is non-nervous, i.e., non-neurofibrillar, is based upon incompletely stained preparations.

3. In stages before protoplasmic connection between tube and myotome is effected certain medullary cells in zones where later the nerve anlagen ('plasmodesmata') make their appearance show in successfully stained Bielschowsky-Paton preparations

a deeply stained neuroreticulum. Similar cells containing a neuroreticulum are the first to become connected by protoplasmic outflow with the adjacent myotome. In all later stages of neurogenesis similar neuroreticular cells within the neural tube are connected by neuraxone processes with the nerve anlage and the myotome. Glia or neurilemma cells participate only secondarily in the formation of neuromuscular connections. The assumption of the Hertwigs ('78), later supported by Paton ('07) and Held ('09), that neuromuscular connections are primarily effected by indifferent cells and that the 'plasmodesmata' thus formed are utilized as 'paths' by the growing neuraxones, does not accord with the observed facts. The term 'plasmodesma' should therefore be discarded as unnecessary and misleading.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

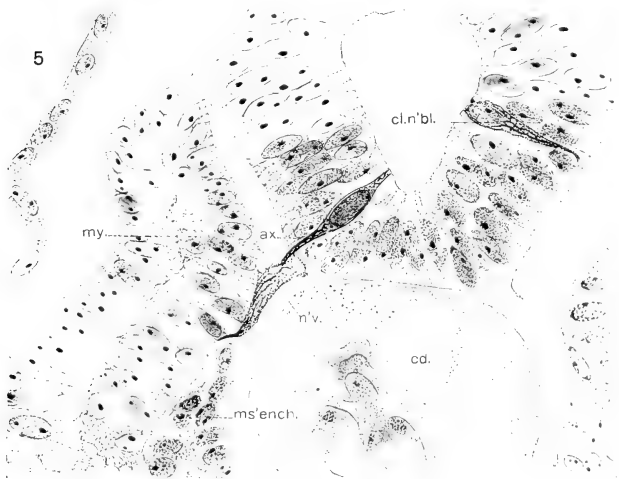
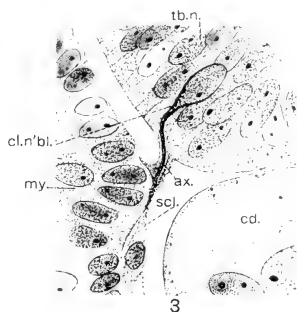
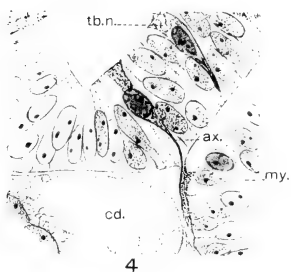
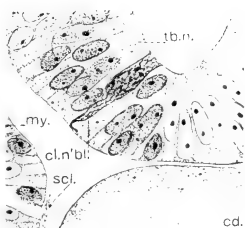
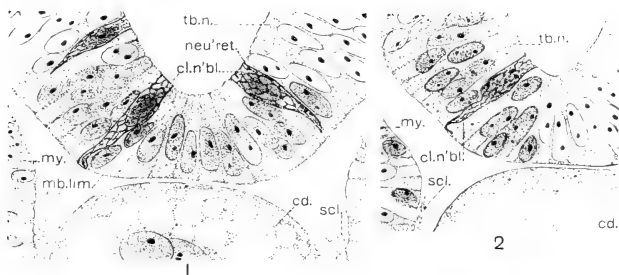
All of the figures of this plate were drawn with Abbé camera, one-twelfth homogeneous oil-immersion objective and no. 6 compensation ocular of Zeiss. In reproduction the magnification has been reduced by one-half. The series illustrates stages immediately preceding and following the appearance of the so-called plasmodesmatous connections between neural tube and myotomes in the trunk region of *Squalus* embryos. In reality such 'plasmodesmata' are the anlagen of somatic motor nerves as demonstrated by their genesis, neurofibrillar structure (when adequately stained) and later histogenesis. As is well known to all students of elasmobranch embryos, there is no difficulty whatever in the determination of stages as earlier and later, since such successive stages appear in successive metameres of each embryo. In all metameres the somatic nerve anlagen make their appearance opposite the middle of the myotome. The embryos from which the drawings were made were prepared by the Bielschowsky-Paton method to demonstrate neurofibrillar structures.

1 and 2 Portions of cross-sections of a 7-mm. *Squalus* embryo cut in the middle of metameres lying near the cloacal region. In the metameres immediately anterior to the ones shown protoplasmic connection between tube and myotome has already been established by the migration of medullary protoplasm. In more posterior segments there is no protoplasmic connection whatever. The sections demonstrate the presence in the medullary wall of neuroblastic cells (*cl.n'bl*) containing a deeply staining neuroreticulum extending throughout the entire length of the cell. Such cells, as stated by the writer in an earlier paper ('14), are the first to effect neuromuscular connections by means of protoplasmic outflow. Their nervous (neuroblastic) character is attested by their polarity and neuroreticular structure even before their neuraxone process extends outside the limiting membrane of the neural tube. They contrast in form, color, and structure with the adjacent cells of the medullary wall. In successful Bielschowsky-Paton preparations the neuroreticulum is stained a deep blue-black, while the cytoplasm of adjacent cells is reddish-yellow in color.

3, 4 and 5 Portions of cross-sections of *Squalus* embryos treated by the Bielschowsky-Paton method in metameres in which neuromuscular connections ('plasmodesmata') are already established. The nervous nature of the so-called plasmodesmata is evidenced by the presence of neurofibrillar material within them and the extension of neuraxone processes of neuroblastic cells into the 'plasmodesmata.' Such evidence shows that instead of being undifferentiated, non-nervous bridges of protoplasm, into which or along which neurofibrils or neuraxones grow in order to effect neuromuscular connection, the 'plasmodesmata' in reality are ab initio nervous structures. Non-nervous material is secondarily added to the nerve anlage by migration or outflow from the neural tube.

ABBREVIATIONS

<i>ax.</i> , neuraxone process	<i>neu'ret.</i> , neuroreticulum
<i>cd.</i> , chorda dorsalis	<i>pl'sm.</i> , plasma
<i>cl.n'bl.</i> , neuroblast cell	<i>scl.</i> , sclerotome
<i>ms'ench.</i> , mesenchyma	<i>so.</i> , somite
<i>mb.cl.</i> , cell membrane	<i>tb.n.</i> , neural tube
<i>mb.lim.</i> , limiting membrane	<i>vac.</i> , vacuole
<i>my.</i> , myotome	



Resumen por los autores, S. E. Johnson y M. L. Mason,
Northwestern University Medical School, Chicago.

El primer ramo comunicante blanco del tórax del hombre.

En los libros de texto de Anatomía humana y en la literatura corriente se encuentran muchas afirmaciones incompletas y contradictorias sobre la presencia de un ramo comunicante blanco en relación con el primer nervio espinal torácico. Los autores del presente trabajo, al coleccionar material fresco en autopsias con el propósito de llevar a cabo un estudio general de la estructura de los troncos del simpático en el hombre, han encontrado en todos los sujetos uno o más ramos comunicantes blancos que reúnen el primer nervio espinal torácico con el primer ganglio torácico o ganglio estelar.

Una disección cuidadosa de doce sujetos dió los mismos resultados (tiñendo y seccionando los ramos, los autores han podido en la mayor parte de los casos distinguir entre los ramos grises y los blancos). Los ejemplares frescos fueron teñidos con ácido ósmico. En un ramo blanco han podido contar un total de 1252 fibras meduladas, que varían entre 1.9 a 15.9 micras de diámetro, la mayor parte de ellas oscilando entre 2 y 4 micras. Teniendo en cuenta el tamaño relativo de las fibras, estiman que por lo menos el 16 por ciento de las fibras meduladas en este ramo eran de carácter aferente.

Translation by José F. Nonidez
Cornell Medical College, New York

THE FIRST THORACIC WHITE RAMUS COMMUNICANS IN MAN

S. E. JOHNSON AND M. L. MASON

Anatomical Laboratory of Northwestern University Medical School¹

FIVE FIGURES

In text-books of human anatomy and in current literature there is evident lack of agreement and uncertainty in the various statements regarding the occurrence of a white ramus communicans in connection with the first thoracic spinal nerve. In Cunningham's text-book of human anatomy ('18) we read that "Each thoracic nerve, with the probable exception of the first, sends a visceral branch (white ramus communicans) to join the gangliated trunk in the thorax." Piersol ('18) says, "from the first or second thoracic to the second or third lumbar." In Lewis' edition of Gray's Anatomy ('18) is this statement: "Two rami communicantes, a white and a gray, connect each ganglion with its corresponding spinal nerve." According to Morris-Jackson ('14), "Each ganglion, with the possible exception of the first, receives a white ramus communicans from a thoracic nerve." The statement as worded does not commit the author as to the source of the white ramus which runs to the first thoracic sympathetic ganglion. It might come from the first, second, or third thoracic nerve, or even from the eighth cervical. In Poirier, Charpy et Cunéo ('08) we find the statement that the thoracic sympathetic ganglia are connected to the thoracic nerves by one or two rami communicantes. This statement is rather typical of the ones found in foreign texts generally. There is seemingly a desire to avoid statements warranting specific interpretation as to the nature of the rami which are described. It is quite obvious that the mere number of rami implies nothing as to their actual nerve constituents.

¹ Contribution no. 81, January 1, 1921.

As further examples of this non-committal sort of statement, we may quote from Chiarugi ('10-'17), "each ganglion is connected by one or two rami communicantes with a thoracic nerve." Similarly in Rauber-Kopsch ('07) no definite statement as to the upward extent of the white rami can be found. Spalteholz ('20) in four separate statements does not attempt to distinguish between the white and gray rami of the thoracic nerves. The following quotation is typical: "Jedes Ganglion (sympathicus) ist durch ein oder mehrere Rami communicantes mit den Nn. thoracales verbunden" (p. 791).

Literature consulted shows that little has been done to determine the nature of the rami communicantes in man, conclusions derived from other mammalian work being read directly into human anatomy. Langley ('94, p. 235) says, "The uppermost white ramus in man should come from the first thoracic, i.e., the first thoracic is probably the highest spinal nerve by which motor and sensory fibers run to the viscera. But it seems not unlikely that a posterior arrangement of the brachial plexus occurs sometimes in man in which the first thoracic has not a white ramus." Langley at another time ('00) states that there is more or less evidence to substantiate the statement that the first thoracic nerve is in all mammals the first to give off a white ramus. And again ('03) he states that afferent fibers which accompany the sympathetic arise from the nerves which give off efferent fibers and from these only, in man they (efferents) arise from the first thoracic to the second or third lumbar.

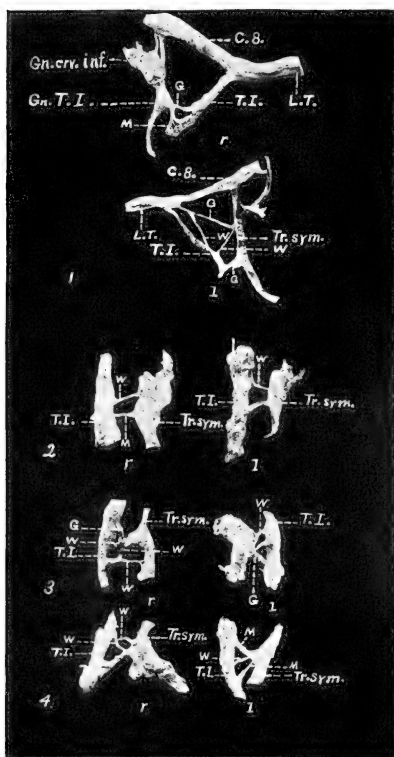
In R. L. Müller's paper ('09) we could find no definite statement as to the superior limit of the white rami. His claim that in all mammals without exception, the inferior cervical ganglion is fused with the first one or two thoracic to form the ganglion stellatum, we are unable to verify as regards man.

In collecting fresh autopsy material for the purpose of making a survey of the structure of the sympathetic trunks in man, the authors found in all cases examined at least two rami communicantes arising from the first thoracic nerve. The number varied from two to five in different subjects and often the number was not the same on opposite sides of the same subject. These

rami did not always run to the first thoracic sympathetic ganglion. They were not infrequently distributed to the inferior cervical (when separate), the second, and even the third, thoracic ganglion.

As a rule the autopsy material had to be removed rather hurriedly, and for this reason it was thought advisable to supplement these findings with careful dissections of laboratory specimens. The first thoracic nerve and its associated rami communicantes were dissected out on both sides of twelve cadavers. The number of rami varied from two to five and was frequently unequal on opposite sides. Examples of the various conditions found are shown in the accompanying photograph, figures 1 to 4. The specimens illustrated were taken from opposite sides of four cadavers. Figure 1, *r*, shows a section of the right sympathetic trunk with the inferior cervical and first thoracic ganglia appearing as distinct enlargements. The internodal segment joining the ganglia, however, was short and thick, and probably contained ganglion cells throughout its length. The eighth cervical spinal nerve (*C. 8.*) is seen to be connected with the inferior cervical ganglion by two rami, medial to its junction with the first thoracic nerve (*T.1.*) to form the lower primary trunk of the brachial plexus. Three rami connect the thoracic nerve with the first thoracic sympathetic ganglion. Figure 1, *l*, from the left side of the same cadaver, shows a more complicated arrangement of the rami. Two run from the eighth cervical nerve to the inferior cervical ganglion, one joins the two nerve trunks, one runs from the first thoracic nerve to the inferior cervical ganglion, and a fifth runs from the first thoracic nerve to the first thoracic sympathetic ganglion. In the remaining specimens shown in the accompanying photograph the inferior cervical and first thoracic sympathetic ganglia are united to form more or less symmetrical enlargements of the sympathetic trunks. Each of these ganglionic masses is connected with its related first thoracic spinal nerve by short nerve strands which vary a great deal in size as well as in number.

Naturally, the presence of two or more rami does not mean anything unless the nature of the fibers which they contain can be determined. Fresh material was stained in osmic acid, and



Figs. 1, 2, 3, and 4 Photograph of right (*r*) and left (*l*) dissections from four cadavers in the anatomical laboratory. In each dissection there are shown short stretches of spinal nerve and of sympathetic trunk with the associated rami communicantes. *C.8.*, eighth cervical spinal nerve; *G.*, gray ramus communicans; *Gn.crv.inf.*, inferior cervical ganglion; *Gn.T.I.*, first thoracic sympathetic ganglion; *l.*, left; *L.T.*, junction of eighth cervical and first thoracic spinal nerves to form lower primary trunk of brachial plexus; *M.*, ramus communicans (mixed) which contains large proportions of both medullated and non-medullated fibers; *r.*, right side; *T.I.*, first thoracic spinal nerve; *Tr.sym.*, sympathetic trunk. Photograph blocked out, but not retouched. Reduced 1/4.

in figure 5 we show a camera-lucida sketch (cross-section) of a first thoracic white ramus communicans which is typical of our series. In the subject from which this section was taken, two well-defined rami ran from the first thoracic nerve to the first

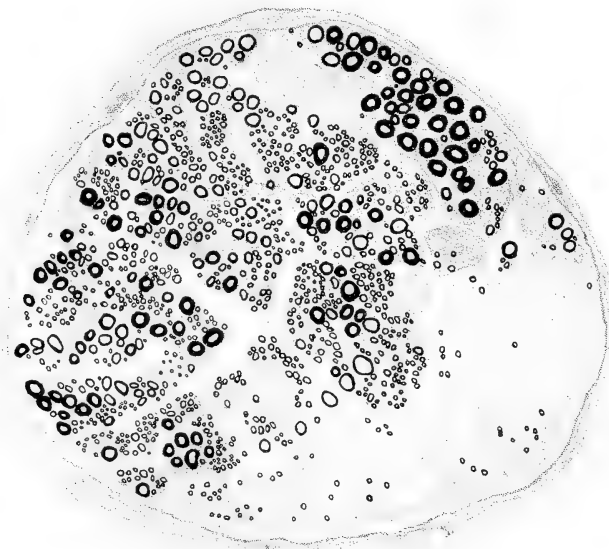


Fig. 5 Transverse section of a first thoracic white ramus communicans. This ramus ran from the first thoracic spinal nerve to the first thoracic sympathetic ganglion. It was removed and fixed in osmic acid two hours after death. Note the separate fascicle of large medullated fibers at the upper margin of the drawing. Camera sketch. $\times 227$.

thoracic ganglion. The latter was connected with the inferior cervical ganglion by a short constricted portion of sympathetic trunk which could hardly be considered an internodal segment. The two rami were of unequal size, the smaller white ramus measuring approximately 0.6 mm. in diameter, and the gray ramus about 1.5 mm. The gray ramus was composed almost

wholly of non-medullated fibers, there appearing only about 150 small medullated fibers, scattered irregularly over the field of a cross-section.

In a cross-section of the white ramus we counted a total of 1252 medullated fibers of various sizes from approximately 1.5μ up to 16.9μ . The results of a differential count may be tabulated as follows:

1.9 μ or less	422
1.9 μ to 3.9 μ	506
3.9 μ to 5.6 μ	151
5.6 μ to 7.5 μ	40
7.5 μ to 9.4 μ	74
9.4 μ to 11.3 μ	35
11.3 μ to 13.2 μ	10
13.2 μ to 15.1 μ	13
15.1 μ to 16.9 μ	1
<hr/>	
Total	1252

In the above tabulation of fibers the feature that stands out most prominently and unexpectedly is the presence of such a relatively large number of sensory fibers. It will be noted that there is an abrupt diminution of fibers of sizes between 5.6 and 7.5 μ . The fibers of this size and under are undoubtedly of two types (Ranson and Billingsley, '18), visceral efferent (the majority) and small afferent fibers. These cannot at present be differentiated microscopically. The remaining 193 fibers range from 5.6 to 16.9 μ , and probably all of these are afferent fibers. Two hundred (approximately 16 per cent) would be a low estimate for the total number of afferent fibers in the ramus. As shown in figure 5, many of the large medullated fibers are grouped in definite fascicles. It will be a matter of the greatest interest to trace out the course and distribution of these well-defined bundles of large medullated fibers.

The rami of the laboratory specimens shown in figures 1 to 4 were run through various fixing solutions, stained in iron haematoxylin, and sectioned, with the hope that we might be able to identify the white rami. This we found to be quite possible in the majority of instances. The nature of the rami is indicated

in the figures (figs. 1 to 4). Rami which were undoubtedly white are marked with the letter *w*. Others are gray (*g*); mixed (*m*), or unidentified as to the nature of contained fibers. In all of the figures with the exception of figure 1, *r*, the first thoracic nerve gives off one or more white rami which in most instances connect with the stellate ganglion. In the case of the specimen shown in figure 1, *r*, the nature of all of the rami could not be determined.

To summarize, we have found, in each of twelve laboratory bodies, as well as in fresh autopsy subjects, one or more white rami communicantes arising from the first thoracic spinal nerve and connecting, as a rule, with the stellate ganglion. These rami contain a relatively high percentage of large medullated fibers. We have not found the inferior cervical and first thoracic sympathetic ganglia invariably fused, as they are stated to be by Müller, although this was the usual condition. Of the numerous rami which we have seen connecting the eighth cervical nerve with the inferior cervical or the stellate ganglion, none have been shown to be either white or mixed in character. However, it does not seem impossible that in an occasional subject the highest white ramus may arise from the eighth cervical nerve or, with a posterior arrangement of the branchial plexus, as Langley has suggested, from the second thoracic nerve. While admitting these possibilities, we feel justified in concluding that the normal condition in man is that which we have described above.

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ganglia, various possibilities have been suggested. These have been considered by Langley ('03), and his observations have been often referred to. Langley has produced much evidence against the existence of such connecting neurons. His results and conclusions which have been reviewed recently by Ranson and Billingsley appear to be supported and confirmed by an increasing amount of purely anatomical investigation. If commissural neurons are present their terminations would be expected to form some part of the intercellular plexus of the sympathetic ganglia, but upon degenerative section of the cervical trunk, Ranson and Billingsley ('18 a) found that the fine axonic ramifications of the intercellular plexus completely disappeared, indicating that it is derived wholly from ascending preganglionic fibers. Two distinct functional types of autonomic cells such as might be anticipated from Dogiel's report could not be demonstrated by Carpenter and Conel ('14) on the basis of cell structure. The writer ('18) has produced experimental evidence against the occurrence of commissural neurons in the sympathetic ganglia of the frog.

Suggesting the occurrence of commissural neurons are observations of a rather fragmentary character which, however, must be considered. The more important of these observations have been reported by v. Lenhossék, Dogiel, and Huber.

Lenhossék's ('94) report is substantially to the effect that he observed, in Golgi preparations of fourteen-day chick embryos, sympathetic fibers entering a sympathetic ganglion from the periphery and there terminating in simple end-brushes. The fibrillae of these end-brushes are said often to terminate on autonomic cells in small end bulbs.

Observations of a somewhat similar nature are recorded by Huber, who, in his 1897 "Lectures on the Sympathetic Nervous System" wrote that "in methylen-blue preparations of the ganglia of the chain taken from mammalia and birds, I have often observed a free ending of branches of non-medullated nerve fibers in sympathetic ganglia." These endings are different from those described by v. Lenhossék, inasmuch as they are on the dendrites of sympathetic neurons rather than on the cell

bodies. Huber states further that the fibers thus ending did not seem to enter the ganglion from the periphery, i.e., did not seem to be terminations of neuraxes whose cell bodies were situated distal to the ganglion under observation. Huber's later observations ('99, '13) do not appear to alter his position with reference to these fibers.

More far-reaching are the observations of Dogiel, who in 1896 described two types of sympathetic cells, 1) the commonly recognized postganglionic motor neuron with cell-body located in a sympathetic ganglion and, 2) another type of cell with usually a larger cell body and much longer dendrites. The dendrites branch less and were often traced beyond the limits of the ganglion. The neuraxes of these cells were sometimes traced into another ganglion, where they were seen to divide repeatedly and take part in the formation of the intercellular plexus. Dogiel expresses the belief that these cells are sympathetic and of sensory function. He refers further to the probability that the pericellular baskets about spinal ganglion cells of type II take their origin from sensory sympathetic cells.

While the observations of the three authors last mentioned have not been materially extended or substantiated by subsequent investigations, it is also true that anatomical and physiological evidence is hardly sufficient as yet to rule out the possibility of the existence of sympathetic commissural neurons.

The writer's contribution to this phase of the structure of the sympathetic trunk is based on an experimental study of the sacral and anterior coccygeal parts of the sympathetic trunk of the domestic cat. As stated previously, this part of the trunk in the cat is especially favorable, as it is connected with the spinal nerves only by gray rami communicantes, the lowest white ramus being that of the fourth or the fifth lumbar nerve (Langley, '92). No preganglionic efferent fibers run from the spinal cord to the sympathetic through the gray rami (Langley, '96). It follows that all preganglionic efferent fibers in the sacral trunk must run through the lower thoracic and lumbar white rami and descend in the trunk to the sacral and coccygeal region. Division of the trunks caudad to the lowest white rami should

therefore produce easily interpreted results, as the field is cleared of the terminations of all preganglionic efferent fibers, leaving only the postganglionic fibers and the terminations, connections, or fibers which are intraganglionic, interganglionic, or reach the trunk by way of the gray rami. The fine axonic ramifications of the intercellular plexus, if formed entirely of preganglionic efferent terminations, should completely disappear, but if formed in part by intraganglionic or other intrinsic commissural connections, these should remain undisturbed. One would also expect marked changes in the internodal segments. As they are largely composed, in the sacral region, of anterior and posterior root fibers, which descend from a higher level, there would be expected to follow practically complete dropping out of the medullated fibers as well as the non-medullated fibers of central origin. The consideration of these facts led to the experiments described in this paper.

MATERIAL AND METHODS

Cats were used for the experiments. In a series of twelve the sympathetic trunks were divided between the seventh lumbar and the first sacral ganglion. For a second series of experiments two cats were employed and each received a double operation. The first operation was the same as that described above. The second operation, performed a week later, consisted of an extensive laminectomy and, in one of the two specimens, removal of the entire cauda equina and conus medularis, and in the other, division of the spinal nerve roots (on the right side) cephalad to and including the seventh lumbar. The dorsal roots were divided distal to the spinal ganglia. All specimens were kept for a degeneration period of from twenty-five to thirty days. At the end of this time the success of the operation was verified and the portions of the trunks under investigation were removed and stained with osmic acid or by the pyridine-silver method. Transverse serial sections were cut at 10μ .

In addition to the experimental material, several normal series of slides of the same parts of the sympathetic trunk were made to serve as controls and for comparison.

The writer is indebted to Dr. P. R. Billingsley for doing the operation on several specimens and for the dissection illustrated in figure 1.

OBSERVATIONS

Normal specimens

As a continuation of the lumbar trunks the sacral portions converge rapidly and lie in close contact with the caudal blood-vessels. Each trunk possesses, as a rule, three ganglionic enlargements, but internodal ganglionic swellings were frequently seen. The first sacral ganglion is the largest of the three and was found constantly present in all specimens examined. The second sacral ganglion is much smaller than the first and occasionally its cells were scattered through the internodal segments so that there was not present any single distinct ganglionic enlargement. The third pair of ganglia was present in all specimens examined and the ganglia were of slightly larger size than the second. The coccygeal trunks were followed caudad to the eighth ganglion and appeared to be more constant in macroscopic structure than the sacral.

The internodal (or interganglionic) segments of both sacral and coccygeal portions of the trunk were found to be most variable as to size and number. In most cases the internodal segment was represented by two or three separate nerve strands and any number from one to five was not infrequently seen. Fusion of opposite ganglia and connection of opposite trunks by nerve strands was found in practically all specimens, and in many cases it was impossible to say with certainty which strands belonged to the right internode and which to the left.

The coccygeal trunks diminish rapidly to mere thread-like filaments, and in their dissection a binocular microscope of low power was a most valuable aid. Gray rami were dissected out as far caudad as the fourth coccygeal ganglion.

The gray rami of the sacral and coccygeal region leave the trunks, as a rule, near the caudal poles of the ganglia, but a good many were seen which followed the internodal segments for half their length or even more before diverging from the trunk.

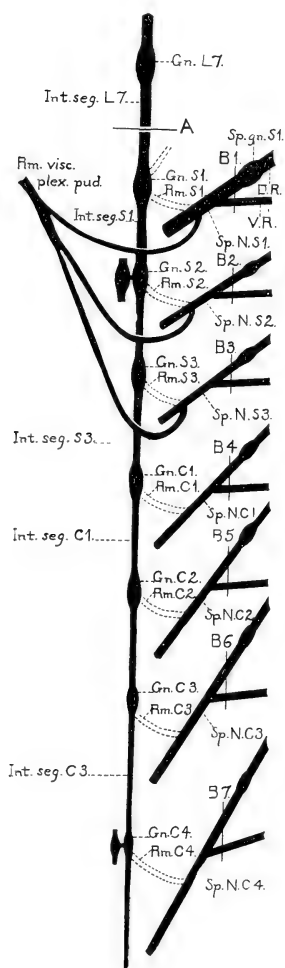


Fig. 1

It is not the writer's purpose to present in this paper a complete account of the normal microscopic structure of the sacral and coccygeal sympathetic trunks. Only observations which are essential to a correct interpretation of the experimental results are included. This applies particularly to fiber counts. The counts given below for normal specimens were not planned to be more than approximately correct. For the purpose of this paper, perfect counts would be no more useful as they would not alter the significance of the experimental results which were obtained.

Three cats were used for normal preparations. The right trunks were stained by the pyridine-silver method and the left were fixed in osmic acid. The preparations of one of these specimens will be described and used as a basis for comparison and contrast with the experimental material.

The ganglia. The sacral and coccygeal ganglia present the same general features microscopically as the ganglia of the more cephalad portions of the trunk. The nerve cells, however, appear to be more irregularly and more widely scattered through the internodal segments. Serial sections show that many internodes contain ganglion cells throughout their entire length. Figure 4 shows a section taken approximately midway between the first and second sacral ganglia and it contains five nerve cells.

The intercellular plexus has been described fully and the literature pertaining to it considered by Ranson and Billingsley ('18 a, p. 337, et seq.). In the writer's preparations of the sacral and coccygeal ganglia this plexus is well stained and shows

Fig. 1 A diagram of the lower part of the sympathetic trunk, the associated spinal nerves, and the gray rami communicantes as far caudad as they were dissected. From a drawing by Dr. Paul R. Billingsley. The line at *A* shows the level of division of the trunk in the first operation. The lines *B1* to *B7* indicate the points at which the spinal nerve roots were divided in the second operation. *Gn.L7*, seventh lumbar ganglion; *Gn.S1* to *Gn.S3*, the sacral ganglia; *Gn.C1* to *Gn.C4*, first to fourth sacral ganglia; *Int.seg.L7* to *Int.seg.C3*, internodal segments from the seventh lumbar to the third coccygeal; *Rm.S1* to *Rm.C4*, gray rami communicantes, first sacral to fourth coccygeal; *Sp.gn.S1*, first sacral spinal ganglion; *Sp.N.S1* to *Sp.N.C4*, spinal nerves, first sacral to fourth coccygeal; *V.R.*, ventral root of spinal nerve; *D.R.*, dorsal root of spinal nerve.

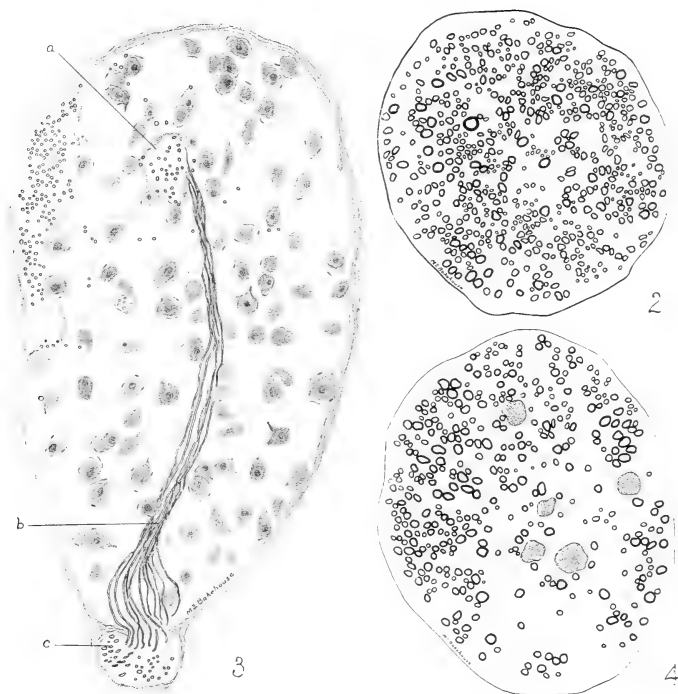


Fig. 2 Transverse section of the seventh lumbar internodal segment of a normal specimen. There is no apparent special grouping of the medullated fibers, and no preponderance of any one size. There are a few fibers less than 1.5μ in diameter, a great many from 1.5 to 7μ , and a small number from 7 to 10μ . Camera sketch. Osmic acid. $\times 440$.

Fig. 3 Transverse section through the first sacral ganglion. Same specimen as illustrated in figure 2. Most of the medullated fibers are grouped in a crescent-shaped bundle in the periphery of the ganglion. A detached fascicle (a) could be traced through the entire ganglion. A fair-sized bundle of fibers (b) runs from fascicle (a) to the forming ramus (c). A few small medullated fibers are seen scattered throughout the ganglion. Osmic acid. Camera tracing. $\times 136$.

Fig. 4 Transverse section of the first sacral internodal segment, approximately midway between the first and second sacral ganglia. Ganglion cells were scattered throughout the length of the internode, and five cells appear in this section. Osmic acid. Camera sketch. $\times 300$.

the same general features. In pyridine-silver preparations the dendrites radiating out from the nerve cells as well as the post-ganglionic axones appear rather lightly stained and can be traced for only a short distance away from the cell body, usually not more than a distance equal to the diameter of a nerve cell multiplied by approximately one to four. The greater part of the plexus is formed by fibers of lesser diameter and of much darker staining properties. The fine axonic ramifications of this plexus give the same appearance as those shown by Ranson and Billingsley ('18 a) to disappear from the superior cervical ganglion upon degenerative section of the cervical trunk. A section of normal ganglion stained by silver nitrate is shown in figure 5.

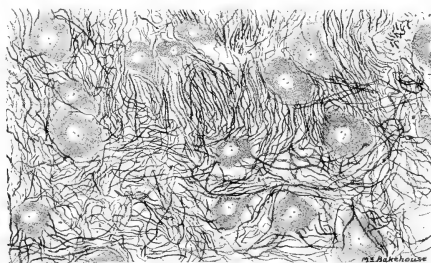
Osmic-acid preparations of normal ganglia show chiefly the variously sized medullated fibers. The majority of these fibers occupy distinct bundles which lie at the periphery of the ganglion. Small medullated fibers (1.5 to 4μ) are scattered throughout the ganglion and occasionally a fair-sized bundle containing larger fibers was seen in the interior of the ganglion. At intervals in serially mounted sections small bundles of variously sized medullated fibers were seen to separate from the main group and form part of the gray rami (fig. 3).

The internodal segments. A considerable number of non-medullated fibers was seen in pyridine-silver sections of the sacral and coccygeal internodes. Small groups of these fibers are scattered irregularly among the medullated fibers throughout the section. Neither the size of the individual groups of fibers nor their distribution appeared to bear any constant or suggestive arrangement in different specimens. The crescent-shaped fascicle observed by Ranson and Billingsley ('18 b, p. 426) in the thoracic trunk does not appear to be present in the sacral and coccygeal portions.

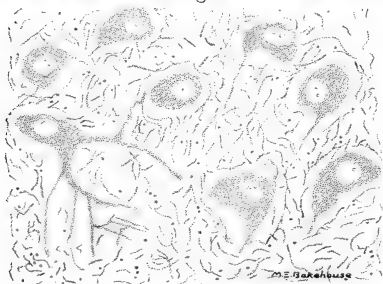
The medullated fibers, afferent and preganglionic efferent, are best seen in sections stained by osmic acid. In the internodes at some distance from the ganglia these fibers are distributed evenly over the field of a cross-section (fig. 2). As they pass through the ganglia the medullated fibers occupy a peripheral position. A gray ramus may be given off from the lower pole

of a ganglion or may arise from the internode somewhat more distally.

The size of the fibers in cross-section varies from 1.5 to 9 or more micra. A small number of medullated fibers less than



5



6

Fig. 5 A small portion of the second sacral ganglion. Normal specimen. The axons and dendrites of the autonomic cells are lightly stained, while the terminations of the preganglionic efferent fibers are finer and much darker. Compare this camera-lucida sketch with figure 6. Pyridine silver. $\times 500$.

Fig. 6 A section of the third sacral ganglion, made thirty days after division of the sympathetic trunk between the seventh lumbar and the first sacral ganglion. The intercellular plexus of fine fibers seen in normal ganglia has completely disappeared. Pyridine silver. Camera-lucida sketch. $\times 500$.

1.5 μ in diameter was seen in the lower poles of the ganglia and in the gray rami. Occasionally a few, never more than four or five, of these very small medullated fibers were seen in an inter-

nodal segment caudad to the origin of the associated gray ramus. Fibers of this type were the only medullated fibers seen in certain of the experimental material.

As to the total number of medullated fibers at various levels in the sacral trunk, the numbers given in the succeeding paragraphs are probably too low, as the oil-immersion lens was not used to check up on the number of the smallest fibers. The enumerations were carefully made under a 4-mm. objective and a 10X-ocular, and the results are sufficiently accurate to serve as a basis for comparison with the degenerated specimens. The figures given are all for the same normal specimen.

In a cross-section of the seventh lumbar internodal segment (i.e., the segment connecting the seventh lumbar with the first sacral ganglion) a total of 444 medullated fibers was counted. Of this number there were counted seven fibers 9μ in diameter, eighty fibers 6 to 7μ , and 357 of various sizes from 1.5 to 5μ . The fibers are evenly distributed over the entire field of the section without any apparent grouping as to size (fig. 2). The very largest fibers, however, appear to be more frequently located near the periphery of the trunk.

Figure 3 shows the arrangement of the medullated fibers as they pass through the first sacral ganglion. There are about 300 medullated fibers in this section, and as the trunk just above the ganglion contains approximately 450, it seems probable that the decrease in number is due to the termination in the ganglion of a proportionate number of preganglionic efferent fibers. The ramus which is seen in the process of formation in this section contains, a few sections caudad where it is completely formed and separated from the trunk, 107 medullated fibers of all sizes seen in the trunk.

In the first sacral internodal segment (fig. 4) were counted 313 medullated fibers, a decrease of 131 from the number in the seventh lumbar segment. This segment was represented by a single nerve strand, but it contained nerve cells throughout its entire length.

Right and left second sacral ganglia were fused together in this specimen, a condition commonly seen in the sacral and

coccygeal region. As in other ganglia, the majority of the medullated fibers are located peripheral to the ganglion cells. The ganglion in this case is larger than usual and apparently receives the terminations of approximately 135 medullated preganglionic fibers.

The second sacral internode consisted of three fine nerve strands with a total of 178 medullated fibers. Sixty-seven of these fibers run into a ramus a short distance caudad to the ganglion.

The third sacral ganglion in this specimen was exceptionally small, producing only a slight enlargement in the diameter of the trunk. The ganglion was fused with its fellow of the opposite side and four nerve strands connected the united ganglionic mass with the first coccygeal pair of ganglia. These four nerve strands comprised the internodal segments of both trunks. Together they contained a total of 254 medullated fibers. This would allow an average of half that number for each internodal segment.

The coccygeal trunks present the same general characters as the sacral. Sections were made to and including the third coccygeal internodal segment, and the outstanding feature in these specimens is the presence of a relatively large number of fibers (sensory) with diameters ranging from 5 to 9 or more micra.

Results of the experiments

As described in a previous section, the experimental material was obtained, 1) by dividing both sympathetic trunks between the seventh lumbar and the first sacral ganglion and allowing twenty-five to thirty days for degeneration to take place before removing the sacral trunk for staining and, 2) by dividing the trunks as stated above and in addition dividing or destroying the spinal nerve roots distal to the spinal ganglia. These animals were kept alive from twenty-five to thirty days from the time of the second operation.

For the first series of experiments twelve cats were operated, one after another as time permitted over a period of almost two

years. In these specimens there constantly appeared a greater number of large medullated (dorsal root) fibers than was anticipated would enter the trunks through the gray rami. This large number of specimens was used to assure the writer that these fibers could not possibly be due to failure of degeneration or to regeneration through union of the divided trunk ends. The results obtained in all of these specimens are characteristically uniform and it will therefore be sufficient to describe the changes from normal in one example.

In the specimen to be described sections were cut serially beginning with the first sacral ganglion, the trunk having been divided just cephalad to the ganglion. The left trunk was stained with osmic acid, the right with silver nitrate.

In the pyridine-silver preparations of the ganglia the nerve cells and their dendrites are stained as in normal specimens, although the dendrites can be followed for a greater distance owing to the absence of axonic terminations. The characteristic change is in the intercellular plexus. The fine axonic terminations seen in normal pyridine-silver preparations have entirely disappeared. The few fascicles of fibers which remain do not stain as darkly as the fibers of the intercellular network and the majority can be traced into the gray rami. A few appear to form small but definite fascicles in the internodal segments and apparently enter successive rami or branches of distribution.

Figure 6 shows a section of sympathetic ganglion after degeneration of the preganglionic fibers. The elimination of the intercellular plexus by section of the sympathetic trunk caudad to the lowest white ramus was anticipated in view of the results obtained by Ranson and Billingsley ('18 a) in the superior cervical ganglion. In osmic-acid preparations, however, the results appeared at first more difficult to explain.

A section through the upper third of the first sacral ganglion showed sixteen small medullated fibers, all under 3.5μ . Near the lower pole of the ganglion a ramus containing eleven medullated fibers of various sizes from 1.5 to 9μ separated from the trunk. There was only one fiber of 9μ in diameter, one 8μ , one 6μ and all of the others were under 5μ .

In the first sacral internodal segment twenty-nine medullated fibers were counted. The proportion of the various sizes was practically the same as given above. A few are scattered over the entire field, but the majority occur in small groups at or near the periphery of the section.

In the gray ramus associated with the second sacral ganglion twenty-five medullated fibers were counted. These are of various sizes from about 1.5 up to 9 or more micra. A cross-section of the second sacral ganglion contained fifty medullated fibers. Most of the fibers are located near the periphery of the ganglion, but a good many of less than 4.5μ in diameter are scattered over the entire section.

Fifty or more fibers were counted in the second internodal segment. Classified as to size, there were two fibers 9μ in diameter, ten fibers 8μ , fifteen fibers 6μ , and the rest ranged from 1.5 to 5μ . All except a few small fibers occupied a crescent-shaped bundle in one side of the trunk.

Caudally in the trunk the medullated fibers decrease slowly in number to the third coccygeal internodal segment (as far caudad as sections were cut) where the number of large medullated fibers varied in different specimens from three to twenty with a somewhat greater number of smaller medullated fibers.

Throughout the trunk the largest of the medullated fibers are seen at the periphery of the ganglia and internodal segments and the majority can be traced into the rami. The smallest of the medullated fibers, many less than 1.5μ , do not appear to have a definite arrangement in the ganglia, but as they enter the internodes most of them join the fascicles of larger fibers and enter the rami. It seems probable that these small fibers are postganglionic axones which have acquired thin medullary sheaths. The large and intermediate fibers remain to be accounted for. Their size and heavy myelinization would appear to preclude the possibility of these fibers being either the axones of intrinsic commissural neurons or the axones of postganglionic nerve cells. Many of the fibers cannot be readily distinguished microscopically from the axones of preganglionic efferent neurons, but the only pathway for anterior root fibers was interrupted by

division of the seventh lumbar internodal segments, since pre-ganglionic fibers are absent in the gray rami (Langley, '96). The most probable explanation for the presence of these fibers appears to be that they are afferent (dorsal root) fibers which enter the trunks by way of the gray rami. Such a large number of dorsal root fibers entering the trunks by this route was not expected, and in order to make certain as to the source of these fibers the second series of experiments was carried out.

In the first cat operated for this experiment both trunks were cut between the seventh lumbar and the first sacral ganglion. A week later the lamina of the sacrum and the sixth and seventh lumbar vertebrae were resected and the entire cauda equina and conus medullaris removed. White ramus fibers descending in the trunk were eliminated by the first operation, while the second operation removed the source of any dorsal root fibers which might reach the trunk through the sacral gray rami. The cat was killed thirty days after the operation. On dissection the trunks and rami were found to be much more reduced in diameter than was usual in the singly operated specimens. The trunks were stained in osmic acid and serial sections cut from the first sacral to the third coccygeal ganglion. A section of lumbar trunk was run as a control and short pieces of the sacral nerves were stained and sectioned in order to check up on the degeneration of dorsal and anterior root fibers in the spinal nerves. These sections showed practically complete degeneration of all medullated fibers.

In a section through the first sacral ganglion there were found only five small medullated fibers ranging from about 1.2 to 2 μ in diameter. They occupy a small part of a fairly large fascicle, the other medullated fibers of which have undergone degeneration. A few sections caudad this fascicle separates from the trunk to form a gray ramus. In sections through the internodal segment just caudad to the ganglion no medullated fibers could be found, although it was expected that a few of the same type as found in the ramus would be present. The conditions found in the rest of the trunk may be summed up briefly in the statement that all large and intermediate fibers as well as the majority

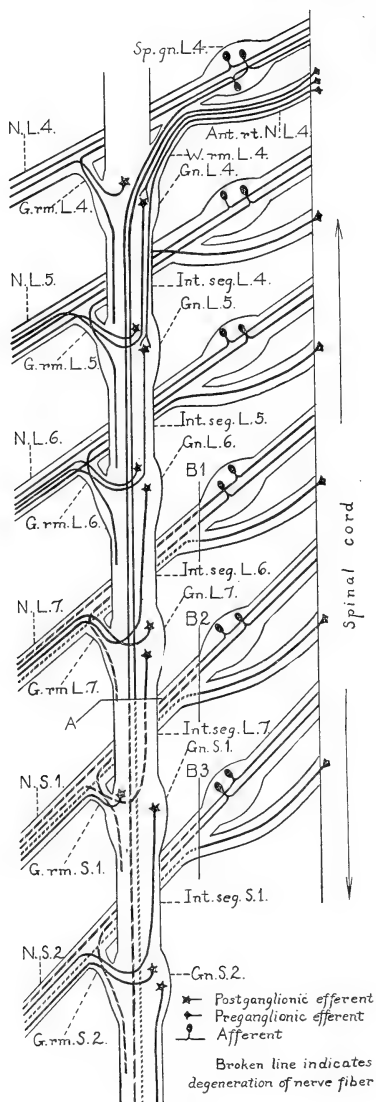


Fig. 7

of the small fibers have completely degenerated, and that only an insignificant number of the smallest medullated fibers found in the trunk remain. The few small medullated fibers which do remain appear to be practically limited to the gray rami and the lower poles of the ganglia. In only one instance (the second coccygeal internode) were any of these fibers found in the inter-nodal segment distal to its related gray ramus.

In a second specimen the operation was modified to include only the spinal nerve roots on the right side in addition to intra-abdominal division of both sympathetic trunks as previously described. Both trunks were stained, and in the right the results are identical to those described above, in the left, to the results obtained in the singly operated specimens, there appearing the usual number of variously sized medullated fibers.

It is hardly necessary to say that in the few ganglia of these specimens which were stained by the pyridine-silver method no trace of the intercellular plexus could be found.

SUMMARY AND CONCLUSIONS

The sacral trunk presents many variations in macroscopic structure. As a rule, it consists of three small ganglionic enlargements, each ganglion connected with an adjacent one by one nerve strand termed by Ranson and Billingsley ('18 a) the internodal segment. The number of ganglia, however, may be reduced or increased or the ganglion cells may be scattered throughout the length of the trunk. Adjacent ganglia are often

Fig. 7 A diagrammatic sketch to illustrate the relations and paths of degenerated fibers in the sympathetic trunks of cats which have been operated upon as described in the text. The broken lines indicate degenerated fibers. *A*, level of division of the sympathetic trunks; *Ant.rt.N.L.4*, anterior root of fourth lumbar nerve; *B1* to *B3*, points at which spinal nerve fibers were interrupted by the second operation; *G.rm.L.4* to *G.rm.S.2*, gray rami communicantes, fourth lumbar to second sacral; *Gn.L.4* to *Gn.S.2*, sympathetic ganglia, fourth lumbar to second sacral; *Int.seg.L.4* to *Int.seg.S.1*, internodal segments, fourth lumbar to first sacral; *N.L.4* to *N.L.7*, fourth to seventh lumbar nerves; *N.S.1*–*N.S.2*, first and second sacral nerves; *Sp.gn.L.4*, fourth lumbar spinal ganglion; *W.rm.L.4*, white ramus communicans of the fourth lumbar nerve. Note that no undegenerated fibers of central origin can reach the sympathetic trunk caudad to the seventh lumbar internodal segment.

connected by two or more strands representing the internodal segment. Fusions between opposite ganglia of the same segment are common. By degenerative section of the trunks and dorsal roots the internodal segments are reduced to the finest thread-like filaments.

The number of medullated fibers contained in the first sacral internodal segment varies a great deal in different specimens. The numbers observed ranged from 400 to 700, although counts were not made with the greatest possible care and a good many of the smallest fibers may have been missed. The fibers are of all sizes from about 1.2 to 9 or more micra in diameter. The majority of these fibers degenerate when the trunks are divided caudad to the lowest white ramus. In view of this fact and other evidence previously considered, it seems practically certain that the fibers in question are dorsal and ventral root fibers which have descended in the trunk from the lower white rami. The very large fibers are obviously sensory fibers from the spinal ganglia. That a good many of the small and intermediate fibers must be from preganglionic efferent neurons is indicated by the disappearance of the intercellular plexus in these specimens. The fibers which remain after the single operation described above number approximately from ten to fifty in different specimens and are of all sizes seen in the normal trunk. Section of the dorsal roots in addition to the first operation caused all of these fibers to degenerate, except an insignificant number which were thinly medullated and mostly under 1.5μ in diameter. All fibers which remain after the first operation except these few very small ones appear therefore to be dorsal root fibers which have reached the trunk by way of the gray rami. Langley ('96) admits the possibility of the gray rami containing a few dorsal root fibers, but such a relatively large number was not anticipated. The small fibers which remain after the double operation do not occur constantly in different specimens nor regularly in successive internodes of the same specimen. Five was the largest number seen in the cross-section of any internodal segment. In most instances they could be traced into the gray rami. It seems probable that these fibers

are axones of postganglionic cells which are in the process of myelinization.

The sacral and coccygeal ganglia possess a rich intercellular plexus which, so far as comparison has been made, does not differ in any essential detail of structure from the intercellular plexus in the cervical and thoracic ganglia. It completely disappears following descending degeneration of preganglionic axones in the lower lumbar and sacral trunk. It must therefore be formed by the terminations of preganglionic efferent axones which run to the trunk through the lower white rami.

Regarding the occurrence of commissural neurons in the sacral and coccygeal ganglia, it is hardly necessary to add that no evidence of the presence of such structures has been found. On the contrary, all nervous elements stained by the methods employed have been traced to some other source or otherwise accounted for. While it may be argued reasonably that possibly there are nerve elements present which do not stain by the methods employed for these experiments, it is to be remembered that such structures have never been satisfactorily demonstrated to exist by any other method. Further, the extensive observations of J. N. Langley have shown that not only are commissural fibers not necessary to explain the physiology of the sympathetic system, but that their presence, in the case of certain limited reactions, would introduce real difficulties for explanation.

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Resumen por el autor, O. Larsell,
Northwestern University.

Terminaciones nerviosas en el pulmón del conejo.

En el epitelio de los bronquios intrapulmonares primarios y en los puntos de división de los órdenes consecutivos de bronquios existen terminaciones nerviosas sensoriales. Los puntos más distales en los cuales se han encontrado son las paredes de los atrios del pulmón. Las diferencias estructurales relacionadas con la posición indican la existencia de tres tipos funcionales de terminaciones nerviosas. Las células ganglionares de los pulmones están rodeadas por redes pericelulares intracapsulares características, que representan los procesos terminales de las fibras nerviosas, originadas en apariencia en el vago. Estas células emiten axones que se distribuyen por la musculatura lisa del árbol bronquial. Existe de este modo una disposición típica preganglionar y postganglionar. La arteria pulmonar y sus ramas, incluso las arteriolas, presentan una rica inervación de fibras amielínicas que terminan en relación con las células musculares lisas de la túnica media. Existen también unas cuantas fibras nerviosas en las paredes de las venas pulmonares.

Translation by José F. Nonidez
Cornell Medical College, New York

NERVE TERMINATIONS IN THE LUNG OF THE RABBIT¹

O. LARSELL

Department of Zoology, Northwestern University, Evanston, Illinois

FIFTEEN FIGURES

The innervation of the mammalian lung appears to be little understood by either anatomists or physiologists. The average text-book description states that the lungs receive their nerve supply from the sympathetic system by way of the pulmonary plexuses, and that the vagus nerve also contributes fibers. Usually references, more or less vague, are made to motor terminations in the bronchial musculature and in that of the pulmonary artery, and also to sensory terminations in the parenchyma of the lung. Physiologists have evidence for the presence of both sensory and motor fibers in the bronchi and their branches, but concerning the vasomotor control of the pulmonary circulation they have obtained discordant results.

It was with the hope of throwing further light on this subject that the present investigation was undertaken at the suggestion of Dr. W. S. Miller, to whom the writer is greatly indebted for assistance with difficult points of the anatomy of the lung.

The general problem undertaken has to do with the distribution and arrangement, as well as the source, of the nerve fibers which enter the lung, in addition to the types of nerve terminations and the position of the latter within the organ. A description of the fibers, their origin and the relation of the intrapulmonary ganglia is reserved for a subsequent report, and these points will be touched upon at present only to the extent necessary to make clear the relations of the various sensory and motor terminations which are described.

¹ Contribution from the Anatomical Laboratory of the University of Wisconsin and the Zoological Laboratory of Northwestern University.

MATERIAL AND METHODS

Rabbits weighing 3 to 4 pounds were used for the greater part of the present investigation. Various modifications of the methylene-blue technique were tried. The best results were obtained by using 0.05 per cent methylene-blue in Locke's solution or in 0.9 per cent NaCl solution. Both Grübler's and Harmer's methylene-blue were employed with about equal satisfaction.

The best staining of the nerve terminations in the bronchial tree resulted from injecting the warm stain into the pulmonary blood-vessels, through the right ventricle. On the other hand, the best preparations showing the innervation of the pulmonary vessels were obtained by filling the lungs through the trachea, by means of a funnel, with the warm stain. The lung was filled to about normal maximum distention, when the stain was introduced through the trachea, or was inflated with air to about the same degree of distention after the stain was injected into the pulmonary vessels, when that procedure was adopted.

Following either method of introducing the stain, the lung was allowed to lie undisturbed *in situ* for ten minutes. In those cases in which the stain had been introduced by way of the trachea, the excess stain was then drained off. In the other cases in which it had been injected into the blood-vessels this was not necessary. The lungs were then alternately inflated with air and deflated, at the rate of twelve to fifteen times per minute. This was accomplished by means of a rubber-bulb hand blower which was connected to a cannula inserted into the trachea by means of a rubber tubing and a Y-glass connection. A short rubber tube with a pinch-cock was attached to one arm of the Y connection. The blower and the cannula were connected by tubes with the other two arms, respectively. Deflation of the lungs was accomplished by opening the pinch-cock and pressing gently with the hand on the outer walls of the thorax. The thoracic cavity had previously been opened to allow of observation of the process of staining.

It was found that twenty to twenty-five minutes was the optimum time for continuing the oxidizing of the stain in the lung tissue in this manner. The lungs were then quickly extirpated and filled with 8 per cent cold ammonium molybdate, to which two to five drops of 1 per cent osmic acid were added per 100 cc. of the ammonium molybdate. The trachea was then ligated and the lungs immersed in a considerable quantity of the same mixture kept at a low temperature. This was allowed to act overnight. The ammonium molybdate was washed out by allowing the preparation to remain for an hour in running tap-water. The lungs were then filled with 95 per cent alcohol, which was changed several times within an hour, and also immersed in alcohol to bring about fixation and hardening of the tissues. This was followed by absolute alcohol for several hours or overnight. The lungs were then cut into pieces 2 to 4 mm. thick, cleared in xylol, and embedded in paraffin. Some of the lungs were treated with 95 per cent alcohol immediately following the ammonium molybdate, without previous washing in water. These showed the atria and air spaces to better advantage, but many of the air passages were filled with a precipitate which often obscured the nerve fibers. Sections were cut at 25 μ to 100 μ and mounted serially by the usual methods. Some were counterstained on the slide with aurantia.

The pyridine-silver method also was tried on the lungs of several kittens, but with unsatisfactory results.

SENSORY TERMINATIONS

Sensory nerve endings occur in the epithelium of the primary bronchi, at the division points of the bronchi of the various orders and also in the walls of the atria. Careful examination of five pairs of lungs prepared by several modifications of the methylene-blue technique failed to reveal any indubitable nerve terminations resembling the sensory type in the walls of the air sacs or pulmonary alveoli. Many of the preparations appeared to be sufficiently well stained to warrant the expectation of finding such endings in the air sacs and alveoli if they are present.

The sensory nerve terminations in the epithelium of the primary bronchi consist of elaborate ramifications of relatively large myelinated fibers (fig. 1). These fibers are given off from the nerve trunks which course along the bronchi, forming a loose longitudi-

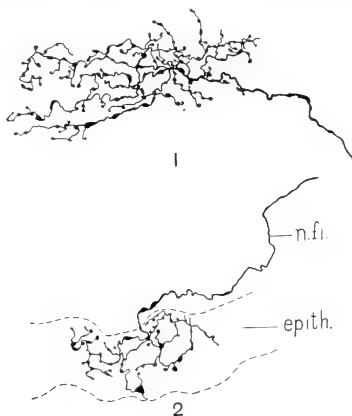


Fig. 1 Sensory nerve terminations in epithelium of primary bronchus within the lung. Rabbit R1. Methylene-blue stain. Camera lucida. $\times 300$.

Fig. 2 Sensory nerve termination at the point of division of one of the larger bronchi: Rabbit R1. Methylene-blue stain. Camera lucida. 50μ . $\times 280$.

ABBREVIATIONS FOR ALL FIGURES

art.pul., pulmonary artery
br., bronchus
brl., bronchiolus
du.alv., ductulus alveolaris
epith., epithelium
l.nod., lymph nodule
lu., lumen
lv., lymphatic vessel

mu.bd., muscle band
mu.c., smooth muscle cell
n.fi., nerve fiber
n.ter., nerve termination
n.tr., nerve trunk
po.fi., postganglionic fiber
pr.fi., preganglionic fiber
t.fi., terminal fiber to muscle cell

nal meshwork on the walls of the air tubes, and pass singly or in small bundles of two or three fibers to the base of the bronchial epithelium. When they occur in strands of more than one fiber, these strands break up near the base of the epithelium into single fibers which supply separate areas.

The individual nerve endings spread over a relatively large area of epithelium. The termination illustrated in figure 1 is represented as viewed somewhat obliquely so that the relative thickness of the epithelium in which it lay could not be indicated in the figure. The greatest breadth of this termination is about $125\ \mu$ and its length about $150\ \mu$. As will be noted in the figure, the main nerve fiber breaks up into seven principal branches, which in turn subdivide into secondary and tertiary twigs. All are studded with varicosities of varying size and form, and the tip of each of the terminal twigs is formed by a small knob-like enlargement. These terminal twigs pass between the cells of the columnar epithelium. Many approach the surface of the epithelium, but most of the twigs appear to end between the epithelial cells. These large, intricate nerve terminations resemble in many respects the endings found by Wolff ('02) in the frog's lung, with the difference that those described by Wolff were found in the interalveolar walls.

The most characteristic position in which sensory endings are found in the rabbit's lung is in the area of epithelium which lies between the bronchi of the various orders at the point of division of the bronchi. The plexiform nerve termination is situated at or near the apex of the point of epithelium in the crotch formed by the branching of the bronchial divisions. The endings which occur in the larger bronchial branches are of relatively larger size than those which are present in the epithelium of the lesser divisions. There does not seem, however, to be any difference in the size or appearance of the nerve fibers which lead to these terminations, no matter where located. With the methylene-blue stain they all show varicosities of varying size and form. These fibers are given off from the nerve bundles which run roughly parallel with the bronchi. In many instances they may be traced individually for considerable distances before terminating in the epithelium (figs. 4 and 5). On reaching the epithelium they break up into a network which interlaces among the epithelial cells, very much in the same manner as the larger endings, above described, in the primary bronchi. The plexus thus formed possesses numerous varicosities, the larger of which

appear to constitute nodal points from which the secondary and tertiary branches are given off. As in the case of the larger terminations found in the primary bronchi, some of the tertiary rami approach the surface of the epithelium, but others appear to end between the epithelial cells. All are characterized by small knobs at their tips. In thick sections of 50μ to 75μ , in which these terminations can be studied to best advantage, the greater number of terminal filaments appear to end between the cells of the epithelium.

With this general description in mind, it will be of advantage to turn attention to the peculiarities of the terminations at the various orders of the bronchial branches. Figure 2 represents a sensory ending located at the point of division of a large bronchus. Comparison of this figure with figure 1, from the epithelium of the primary bronchus, will show many points of similarity between the two. The termination from the bifurcating portion of the bronchus has the same characteristic type of ramification, its rami subdivide similarly, and in general have a similar appearance. The principal difference between the two consists in the smaller size of the termination from the smaller bronchial division.

These endings in the larger bronchi appear to correspond with the terminations described and figured by Berkley ('93) as brought to view by the Golgi method of staining. Berkley also figured enlargements on the fibers leading to some of the nerve endings, and suggested that these might be nerve cells. Similar enlargements have been observed in the present study, but they appear to be unusually large varicosities rather than nerve cells. This conclusion is based on the fact that the enlargements in question are of considerably smaller size than any undoubted nerve cells encountered in the lung. Also no nucleus was observed in any of the enlargements of this type encountered, although the method of staining employed would ordinarily differentiate between the nucleus and the cytoplasm, while the Golgi method might not do so.

A point of considerable interest in connection with the nerve terminations at the points of branching of the larger bronchi

consists in the fact that masses of lymphoid tissue are often located between the bifurcating branches in such a manner as to lie in close juxtaposition to the epithelium in which the nerve endings are found. In many cases the fibers leading to the nerve terminations pass through the borders of the lymphoid mass, and the latter frequently extends to the base of the epithelium.



Fig. 3 Sensory nerve termination at the division point of a small bronchus. Rabbit R1, series D5. Methylene-blue stain. Camera lucida. 62μ . $\times 374$.

As the bronchial tree is followed to its smaller branches, it is easy in thick sections to trace individual fibers which are given off from the nerve bundles. These pass either individually or accompanied for greater or shorter distances by one or two similar fibers, and make their way to the points of division of the bronchioli and alveolar ducts. Such a fiber, with its termination at the division of a small bronchus, is illustrated in figure 3. It will be noted that the termination, which is located in a



Fig. 4 Sensory nerve termination at point of division of a small bronchus into two divisions which appeared to be respiratory bronchioli. The drawing was reconstructed from two sections cut at 60μ . Rabbit R1, series C2, Methylene-blue stain. Camera lucida. $\times 374$.

mass of low columnar epithelium, shows much more limited branching than do the two previously described. The general type, however, appears to be the same. There is adaptation to the more limited area of epithelium, and in this case, as in those previously described, the terminal twigs radiate into the surrounding territory of the epithelium.

A termination of somewhat different appearance is illustrated in figure 4. In this instance, and in others resembling it, the

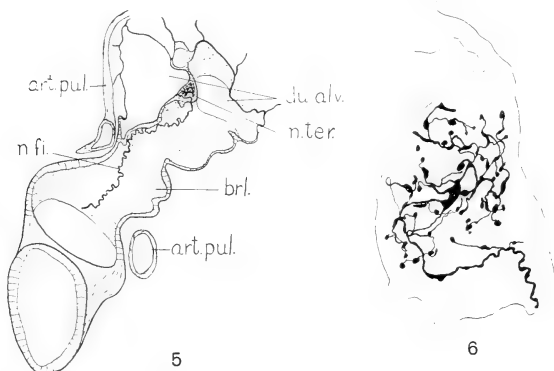


Fig. 5 Graphic reconstruction of two sections cut at 62μ , illustrating the relation of a nerve fiber and its sensory termination to a respiratory bronchiole and two alveolar ducts which arise from the bronchiole. Rabbit R1, series D4. Methylene-blue stain. Camera lucida. $\times 60$.

Fig. 6 The nerve termination represented in figure 5, highly magnified. Camera lucida. $\times 660$.

terminal arborization presented as a whole an ovoidal appearance with the ultimate branches curved. These terminal twigs were found in a nodule-like mass of epithelium, which was located at the point of bifurcation of a small bronchus into a respiratory bronchiolus.

In figure 5 is represented a graphic reconstruction from two sections of a series cut at 62μ , to illustrate the relation of a fiber and its termination to a respiratory bronchiole and two alveolar ducts which arise from the latter. The wall of the bronchiole

is represented as translucent, as it appears in a balsam mount, so that the nerve fiber shows through it. The fiber pursues a tortuous course outside the epithelial wall of the bronchiolus to the point where the latter divides to give rise to the alveolar ducts. At this point, between the openings into the alveolar ducts, there is a nodule of somewhat thickened epithelium. The nerve fiber enters this nodule and breaks up into numerous fine branches, each of which terminates as a small knob. All the twigs, as well as the entering fiber, have numerous varicosities.

This same termination is represented at greater magnification in figure 6, in which the details of structure are more clearly shown. As represented in the figure, a central filament may be followed for a considerable distance. This filament gives off the numerous branches to which reference has already been made. These branches interlace in the most confusing manner. Many of them subdivide into smaller rami which terminate in minute knobs. The larger mass near the center of the figure indicates a varicosity of unusual size, which is somewhat foreshortened in the figure, and on that account appears larger than it does in the preparation itself.

The terminal fibers in the two terminations last described, and in numerous others which were observed to occupy a corresponding position, are curved, as already noted, and appear somewhat drawn together, in contrast with the extended and radiating ultimate processes of those previously described. Whether or not this difference is of significance is problematical. The suggestion presents itself that these terminations located at the openings into the respiratory portion of the lung may represent a somewhat different functional type than those located in the larger air passages. It appears possible that the latter represent a type of tactile terminations which may be stimulated by foreign bodies or by masses of mucus within the bronchi. Their location at the portals to the smaller air passages appears admirably adapted to guard against the entrance of such objects from the larger bronchi by causing stimulation at the points such objects would naturally strike, and thus produc-

ing reflex contraction of the bronchial musculature, constricting the smaller air passages still further, and possibly also initiating reflex coughing to expel the object.

The terminations at the openings into the respiratory portion of the bronchial tree might likewise serve the purpose of guarding against the entrance of foreign objects into the atria and air sacs by initiating reflex constriction of the small sphincter-like muscle bands at the openings into the atria. The difference in form, however, of these endings, which appear better adapted to react to pressure stimuli than to touch, suggests the possibility that such terminations may be stimulated rather by the partial collapse of the lung during expiration. When the lung is distended the alveolar ducts are doubtless spread apart at relatively greater angles with each other than are the other subdivisions of the bronchial tree. On expiration they probably approach relatively closer together, thus affording sufficient pressure to stimulate the terminations in question.

The most distal point at which nerve endings have been found in the epithelium is just inside the atria as they are entered from the alveolar ducts. These terminations (fig. 7) are of small size and are covered by a delicate capsule, formed in part by the squamous epithelium, beneath which they lie in the wall of the atrium. The nerve fibers leading to these terminations are branched, as shown in the figure, so that each fiber probably terminates in a number of sensory endings. On entering the capsule the fiber divides into several branches, which in turn subdivide into small twigs. The latter terminate in small knobs. All the branches have typical varicosities. The whole structure has a flattened appearance, roughly elliptical in outline.

It appears possible that this type of termination may be influenced by stretching of the atrial walls during inspiration, and may represent the nerve terminations which many physiologists have maintained are responsible for the inhibition of the inspiratory movements and excitation of expiration.

Retzius ('93) found fibers and terminal branches in a human foetus of 15 cm. reaching to "the necks of the alveoli," as a rule, with an occasional fiber spreading over the rounded alveoli.

Ponzio ('06) has described and figured fibers in the lung parenchyma which he designates as interalveolar, intercellular, and intracellular nerve filaments, respectively, and he also describes an intracellular nerve reticulum. Ponzio employed the

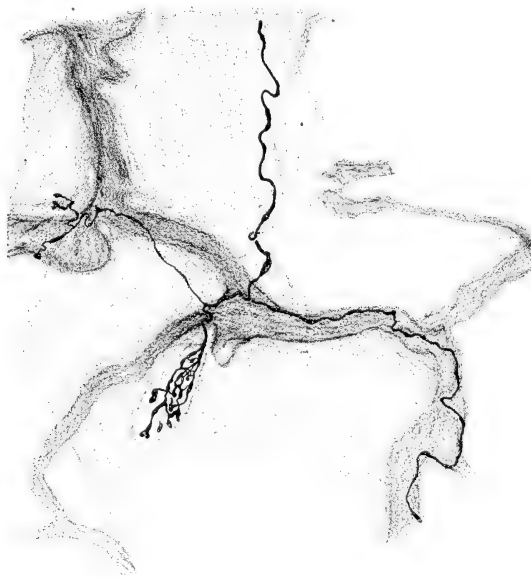


Fig. 7 Sensory nerve terminations in the wall of an atrium. Rabbit R1, series E3. 62 μ . Camera lucida. \times 880.

methylene-blue and Cajal methods of staining on the lungs of newborn kittens and puppies.

In my preparations, both those stained with methylene-blue, and those prepared by the pyridine-silver method, there exist fibers and filaments which closely resemble those illustrated by Ponzio and in corresponding positions. I am inclined to believe that they are elastic fibers rather than nerve fibers. This

conclusion is based on a detailed comparison of such fibers, as demonstrated in methylene-blue and silver preparations of the lung, with the elastic fiber network brought to view by the specific elastic fiber stains, such as orcein and resorcin-fuchsin. Undoubted nerve fibers elsewhere in the lung, even those of the smallest size, have a different appearance from the fibers under consideration. The difference consists in the much darker stain which the nerve fibers take and in the fact that the latter always present varicosities and have a much more irregular course than do elastic fibers.

A reexamination of my preparations after reading Ponzio's article, and a comparison of his figures with the fibers in these preparations, has strengthened the conclusions already reached, namely, that the network of delicate fibers in the walls of the air sacs is composed of elastic fibers. Ponzio's figure 9 should have separate comment and will be referred to again in connection with the innervation of the pulmonary blood-vessels.

There appears little room for doubt that the several terminations described are of sensory type. Their position in the epithelium, particularly at the points which appear most subject to irritation, their connection with the relatively large, myelinated nerve fibers, and the character of their terminal arborizations, appear to point to the conclusion that they are sensory rather than motor. The present writer has not attempted any experimental studies to test these conclusions, but the results obtained by Molhant ('13), who studied the distribution of the sensory fibers of the vagus nerve in the rabbit, agree very well with the interpretation expressed.

Molhant extirpated the superior and median lobes of the right lung in the rabbit, and after allowing time for the injury of the nerve fibers involved in the trauma to affect the cells of origin of these fibers, he stained the peripheral ganglia of the vagus by the Nissl method. He found that ganglionic cells in the external portions of the median and inferior segments of the ganglion nodosum showed the typical degeneration of the Nissl granules as a result of the injury suffered by their peripheral processes. Molhant concluded that the cells thus affected

were the ganglionic cells of the sensory fibers which supply the two lobes of the lung in question.

Similar results had previously been obtained in the dog by Ikegami and Yagita ('07), also by experimental methods. They concluded that from about 12 per cent to 13 per cent (1/8.3 to 1/7.5) of the total number of cells of the right nodose ganglion are in connection, in the dog, with the pulmonary parenchyma.

Chase and Ranson ('14), who studied the finer structure of the vagus nerve in the dog, cat, rabbit, rat, and human, state that the bronchial rami of the vagus contain large numbers of myelinated fibers and take from the vagus a considerable proportion of the myelinated fibers which have continued in it down to this level. They do not make any specific statement as to the size of the myelinated fibers in the bronchial rami of the vagus, but in a preceding paragraph they state that this nerve, just above its pulmonary branches, contains a few myelinated fibers of large size, although the majority are of small or medium size. Below the pulmonary rami of the vagus nerve relatively few myelinated fibers are present.

The results of these various investigators indicate that peripheral fibers from cells in the ganglion nodosum pass by way of the vagus nerve and its pulmonary rami into the lung. It appears reasonable to assume that the sensory terminations above described are the terminal processes of these fibers.

In addition to the sensory terminations in the bronchial tree, the writer is able to confirm the observations of Schemetkin, reported by Dogiel ('98), that there are sensory terminations in the walls of the pulmonary artery. So far as the writer has observed, these are found only near the base of this artery, close to the hilum of the lung.

MOTOR TERMINATIONS

Motor terminations are found in the rabbit's lung, both in the smooth muscle of the bronchial tree and in the muscular coat of the pulmonary vessels.

Pleschko ('97), by staining with methylene-blue, found motor terminations in the smooth muscle of the trachea. He also figured and described ganglion cells with processes which pass to the smooth muscle bundles, there to divide into fine fibers which terminate in the individual muscle cells. These ganglion cells are surrounded by pericellular plexuses of fine varicosed fibers formed by the splitting up of nerve processes which apparently are given off from the vagus nerve. There is present, therefore, in the trachea the typical relation of preganglionic (vagus) fibers to the terminal ganglionic cells with their postganglionic fibers. The latter are represented by the axones which pass to the trachealis muscle, there to ramify in the manner above described.

According to Miller ('18), the musculature of the bronchi is a direct continuation of the musculature of the trachea. It will appear that the same pattern of innervation also holds for the smooth muscle of the bronchi and their branches that is present in the trachea.

Clusters of ganglionic cells (figs. 8 and 9) are found along the primary bronchi and their larger branches. These clusters constitute small ganglia which are usually located along the course of the larger nerve bundles on the wall of the bronchial tree. The larger clusters of cells, which are located nearer to the hilum of the lung, are surrounded by a connective-tissue capsule. This capsule also contains frequently a fairly large nerve trunk, which is composed of both myelinated and unmyelinated fibers. The myelinated fibers are of three sizes, corresponding to the description given by Chase and Ranson ('14) of the fibers in the vagus nerve. There are a few myelinated fibers of large size and more numerous myelinated fibers of small and medium size.

It has been long known that in the walls of the larger bronchi the muscle occurs in bands. In the smaller subdivisions where no plates of cartilage are present, the muscle is said to constitute a continuous sheet. No muscle fibers are found distal to the alveolar ducts, but there is a sphincter-like band at the extremities of these ducts, surrounding the openings into the atria, as was

pointed out to me by Doctor Miller. These various muscle bands have a very rich innervation. The fibers which pass to them are easily distinguished from those which have been described above as sensory fibers, by reason of the smaller size of the former and the fact that they are unmyelinated.

Many of these fibers are direct processes from the ganglionic cells, as shown in figures 8 and 9, and are therefore postgan-

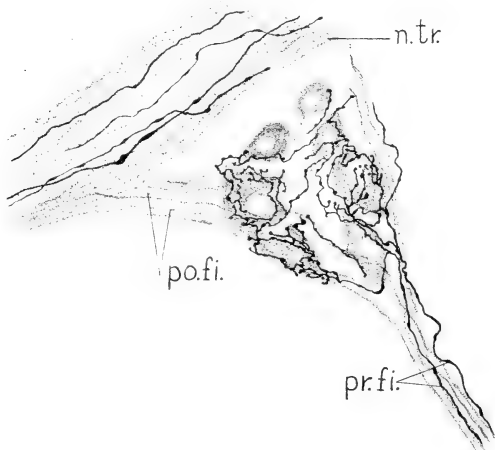


Fig. 8 Cluster of ganglionic cells in the wall of one of the larger intrapulmonary bronchi, showing preganglionic fibers terminating about the nerve cells in pericellular, intracapsular networks, and postganglionic fibers as processes from the nerve cells. A portion of a nerve trunk is also shown. Rabbit R3. Methylene-blue stain. 50μ . Camera lucida, stage level. $\times 400$.

glionic fibers. Some, as in figure 9, can be observed to pass directly from such a ganglion cell to the smooth muscle bands, where they branch into numerous slender varicosed rami. These rami run parallel to the smooth muscle bands between the smaller bundles of muscle cells which compose the bands. At intervals they give off short twigs which terminate as small knobs on the muscle cells. The processes from other ganglionic cells, especially

from clusters of such cells which lie along the nerve bundles, become a part of these nerve bundles and cannot be followed individually. There can be no doubt, however, that they have a distribution and termination similar to that just described for the processes of the relatively few isolated cells which it was possible to follow.

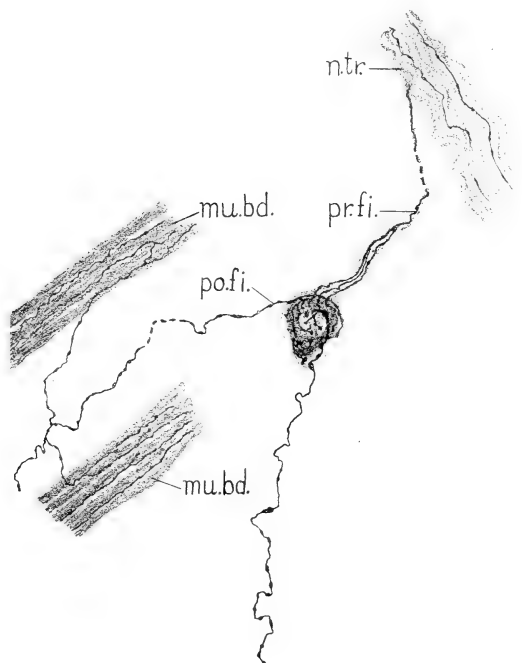


Fig. 9 A single nerve cell in the wall of an intrapulmonary bronchus, showing a fiber (preganglionic) from a nerve trunk, terminating about the cell as an intracapsular, pericellular network, and also showing the axone from the nerve cell. The axone divides into two branches outside the capsule of the cell, each branch passing to bundles of muscle. The manner of termination of one of these branches in relation to two small muscle bundles, is indicated in the left-hand side of the figure. The preganglionic fiber and one of the branches of the axone of the cell have been fore-shortened, as represented by the broken lines, to save space. Rabbit R3. Methylene-blue stain. 50μ . Camera lucida. $\times 400$.

At intervals small bundles of fine fibers are given off from the main nerve trunks. These bundles pass to the muscle bands and there break up by the separation from them of the individual nerve fibers, as shown in figure 10. The individual nerve fibers, on reaching the muscle bands, divide into numerous slender filaments which run parallel between the muscle fibers, as illus-

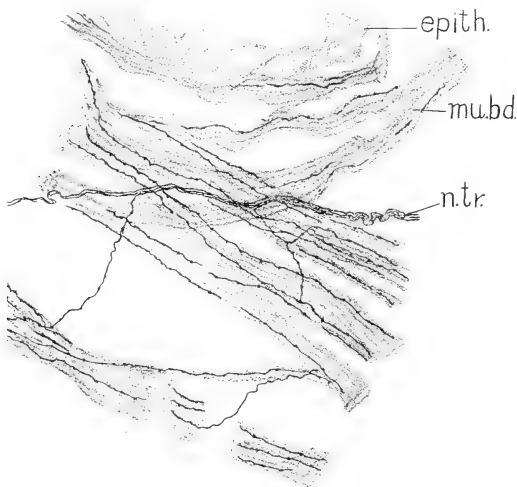


Fig. 10. Muscle bands in wall of a bronchus, illustrating the manner in which the nerve bundles break up into single strands and the manner in which these strands are distributed to the muscle fibers. Rabbit R4. Methylene-blue stain. 60a. Camera lucida, stage level. $\times 150$.

trated in the figure, and at intervals give off short twigs, better shown in figure 11, which terminate near the nuclei of the individual smooth muscle cells, as already described.

The recent work of Carlson and Luckhardt ('20) has demonstrated the presence of inhibitory fibers only, in the lungs of the axolotl and *Necturus*, while both inhibitory and motor fibers reach the lungs through the vagus nerve in the frog. There appears to be an even larger number of motor fibers in the reptilian lung, as represented by the turtle.

If these results can be applied to the mammalian lung, one might expect to find two physiological types here also. It appears possible that many of the fine nerve fibers which are present in the intrapulmonary nerve trunks are not related to the intrapulmonary ganglia, either as preganglionic or post-



Fig. 11 Muscle bands from a bronchus, under higher magnification, illustrating the manner in which the nerve fibers terminate. Rabbit R1, series A1. Methylene blue, with aurantia counterstain. 25μ . Camera lucida. $\times 560$.

ganglionic fibers, but represent fibers from some source outside the lung, which reach the lung by way of the vagus nerve and are distributed to the smooth muscle within this organ. This supposition may serve to account for the very rich innervation of the muscles of the bronchi, particularly. This innervation is much richer than the processes from the intrapulmonary ganglionic cells would appear to account for.

The nerve bundles follow the bronchi in diminishing size to the bronchioli and even to the alveolar ducts. They give off branches which are distributed to the smooth muscle bands around these smaller air passages, as shown in figure 12, which

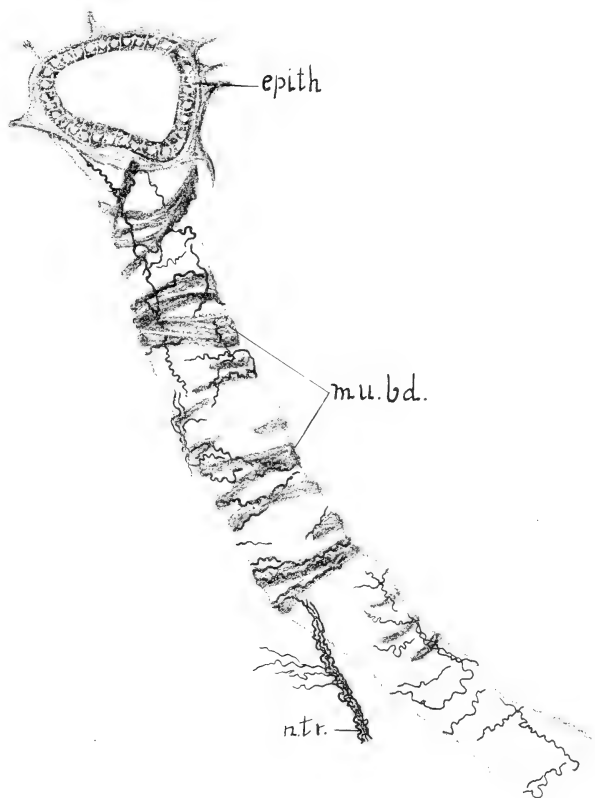


Fig. 12 Nerve terminations on the muscle bands of a bronchiolus. Rabbit R1, series A1. Methylene blue, aurantia counterstain. 25μ . Camera lucida. $\times 300$.

represents one of the smaller bronchioles. The most distal point to which fibers of the motor type have been followed is represented by the small sphincter-like muscle bands at the openings into the atria from the alveolar ducts. Slender varicosed fibers have been observed to run among the muscle cells of these bands and to terminate in relation to them.

No ganglionic cells have been found beyond the larger bronchi, so that the nerve bundles which pass to the smaller air passages, must contain, in addition to the few sensory fibers, which can be recognized by their large size, also the motor and inhibitory fibers.

Innervation of the pulmonary vessels

The pulmonary artery and the vessels branching from it have a much richer nerve supply than the usual anatomical description indicates or than might be inferred from the meager and conflicting results obtained in the past by physiological experiment.

Near the hilum of the lung relatively large nerve trunks accompany the larger pulmonary arterial branches. No ganglion cells have been encountered anywhere along the nerve trunks which accompany these vessels. The source of these nerve fibers is reserved for further investigation. It should, however, be stated at this point that the smaller branches of the pulmonary artery, which lie in close juxtaposition to small bronchi, receive fibers from the nerve plexus around the latter. A similar observation was made by Berkley ('93) in the gray rat. The recent physiological results of Carlson and Luckhardt ('20) indicate that these fibers are derived in large part, if not entirely, from the vagus nerve, at least in Amphibia and reptiles.

The nerve bundles wind about the blood-vessels, giving off individual fibers at more or less irregular intervals. These fibers run roughly parallel with the artery, then turn nearly at right angles to the longitudinal axis of the latter, and divide into several main branches, as illustrated in figure 13. One of these rami usually runs back along the artery, proximally, from the point of primary division of the fiber, another runs distally from this point. These larger branches give off numerous

slender varicosed fibers which in turn subdivide. These apparently correspond to the fibers observed and figured by Karsner ('11) in the pulmonary artery of the dog. The final fibers pass between the smooth muscle bands of the tunica media of the arterial wall, and give off twigs which terminate in relation to the smooth muscle cells, as above described and as illustrated in figure 15, which represents a portion of the wall of an arteriole.

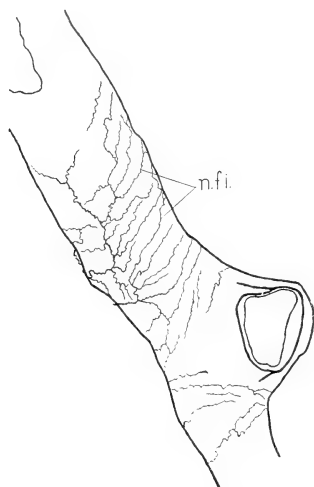


Fig. 13 Distribution of nerve fibers in the muscular portion of the wall of the pulmonary artery within the lung. Rabbit R3. Methylene-blue stain. 50μ . Camera lucida. $\times 75$.

Nerve fibers are present in the walls not only of the larger vessels, such as represented in figure 13, but also in the various subdivisions of the pulmonary arterial system, including the arterioles. This may be seen by examination of figure 14, which represents several orders of branches of a pulmonary vessel, as shown in a single section. That the blood-vessel is a branch of the pulmonary artery can be judged from its relation to the bronchus which is also shown in the figure. W. S. Miller has

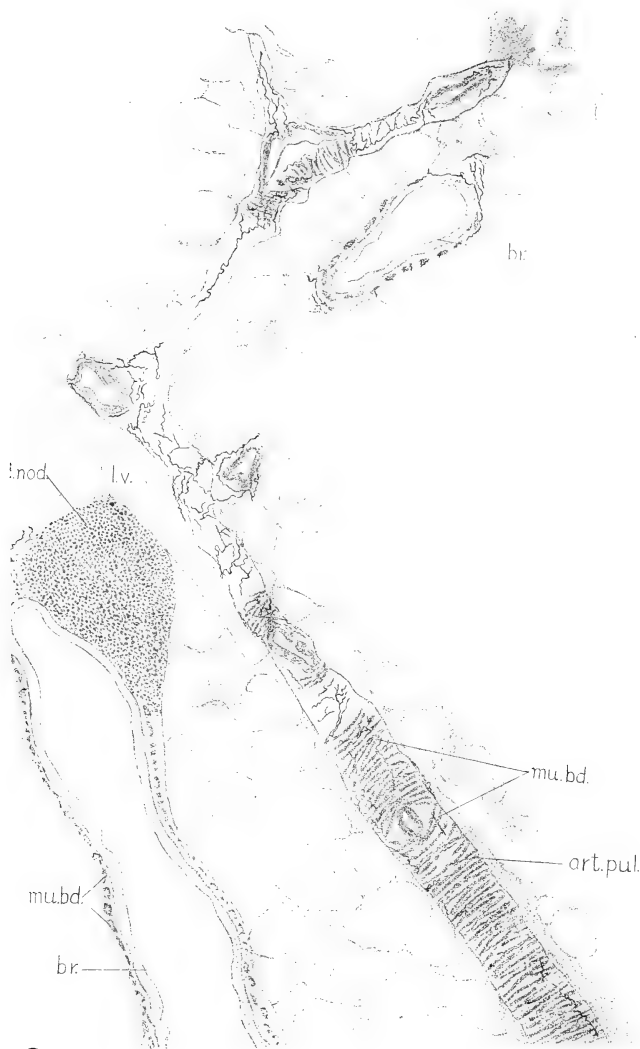


Fig. 14 Pulmonary artery and branches, showing the relation of nerve fibers to the muscular coat of the various branches of the blood-vessel. Rabbit R1, series B2. Methylene blue, with aurantia counterstain. 60 μ . Camera lucida. $\times 60$.

repeatedly emphasized the fact that the pulmonary arterial branches are located "as near as possible to the bronchi" which they accompany, while the corresponding veins are as far removed as the architectural plan of the lung will permit.

It will be observed that the nerve fibers are present all along the larger arterial branch represented, as well as in the various divisions of this branch. The definite relationship to the smooth muscle bands of the arterial walls is not so clearly indicated in all parts of the figure, but this is due to the variations of different portions of the arterial wall with respect to the plane of section.

That these fibers terminate in relation to the smooth muscle cells may be seen from figure 15, which pictures a portion of an arteriole under high magnification. It will be noted that the nerve fiber, which lies in the adventitious layer of the arteriole, gives off lateral branches at short intervals. These branches subdivide and send terminal twigs to the individual smooth muscle cells of the muscular layer of the arteriole. These terminal twigs end as small knobs, apparently on the surface of the muscle cell and usually near the nucleus. In the figure, which was sketched from a relatively thick section, the smooth muscle cells are represented at various angles. Some were cut transversely, some obliquely, and others curve upward on one side of the arteriole, pass across the field and curve downward on the opposite side.

In some of my preparations there are indications of a very delicate nerve plexus on the blood capillaries which are located in the walls of the air sacs and the atria. Extremely fine varicose fibers are present which both in their staining reaction and in their general appearance bear a closer resemblance to nerve fibers, aside from their small size, than to elastic fibers. No indication of nerve cells in connection with this possible plexus was observed, such as have been described by Dogiel ('98) and by Prentiss ('04).

Attention has been already called to Ponzio's figure 9, which pictures a similar network on a blood capillary in the lung. The further study of these fibers appears to the writer to present a separate problem, which must be left for further investigation.

A few nerve fibers were observed in the tunica media of the pulmonary veins. Berkley ('93) made a similar observation. They appear to have the same relation to the musculature of the veins as the fibers above described have to the arteries.

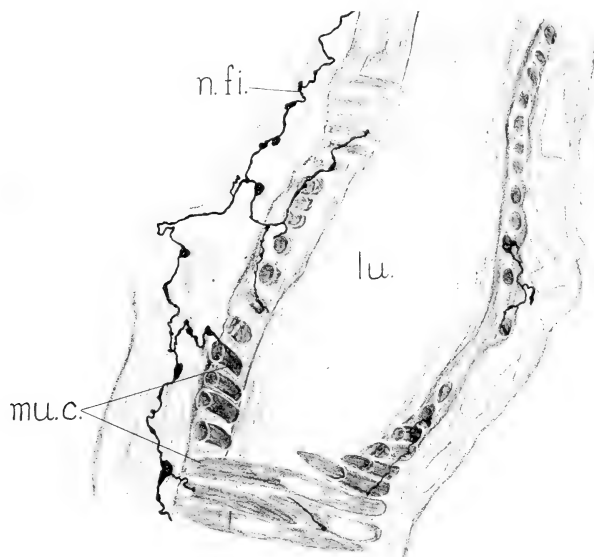


Fig. 15 Arteriole from pulmonary artery, showing a single nerve fiber in the adventitia which gives off short branches which terminate in relation to the individual smooth muscle cells of the tunica media. Rabbit R1, series C3. Methylene blue, with aurantia counterstain. 25μ . Camera lucida. $\times 880$.

SUMMARY

1. Sensory nerve terminations are present in the epithelium of the primary bronchi within the lung and at the points of division of the succeeding orders of bronchi. The most distal points at which they have been observed are in the walls of the atria of the lung.

Differences in structure, correlated with position, suggest three functional types of sensory terminations.

2. Nerve endings, probably motor and inhibitory, are present in the smooth muscle fibers of the bronchial musculature.

3. The ganglionic cells of the intrapulmonary ganglia are surrounded by intracapsular, pericellular networks which are the terminal processes of nerve fibers, apparently of vagus origin. These cells give off axones which are distributed to the smooth muscle of the bronchial tree. There is a typical preganglionic and postganglionic fiber arrangement.

4. The pulmonary artery and its branches, including the arterioles, have a rich innervation of fibers which terminate in relation to the smooth muscle cells of the tunica media.

A few nerve fibers are also present in the walls of the pulmonary veins.

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Resumen por el autor, E. C. Case,
University of Michigan.

Sobre un vaciado endocraneal del reptil triásico *Desmatosuchus spurensis*, del triásico superior del occidente de Texas.

El vaciado endocraneal descrito en el presente trabajo corresponde a un individuo de un nuevo subórden de reptiles, el de los *Desmatosuchia*, descrito en un trabajo preliminar publicado en el *Journal of Geology*, vol. 28, Núm. 6, 1920. Mediante un vaciado, el autor ha conseguido obtener una reproducción muy perfecta de la cavidad endocraneal. El vaciado demuestra la existencia de una epífisis o paráfisis casi vertical, con procesos laterales que se extienden de la misma región cerebral. Los procesos laterales son los vaciados de dos fosas profundas, tal vez orificios, que marcan la pared interna de la caja cerebral a cada lado de la profunda fosa que deprime la pared del cráneo en la posición del orificio pineal, sin llegar a perforarle.

La hipófisis es muy grande y está ligeramente inclinada hacia atrás; la porción anterior no es visible por no estar osificadas anteriormente las paredes de la caja cerebral. Apenas existe una expansión de los lóbulos cerebrales y no se presentan estructuras que indiquen la posición de los nervios ópticos y tálamo. El tracto olfatorio es voluminoso y ancho; los nervios II, III y IV pasan a través de escotaduras formadas por la aproximación de los alisfenoides u orbigitosfenoides; el nervio V sale al exterior por un ancho orificio de las paredes laterales de la caja craneal y no se divide hasta después de abandonar esta. El nervio VI pasa exteriormente a través de la base de la caja craneal. La región ótica está indicada solamente por un proceso, puesto que las otras estructuras fueron destruidas por la fosilización. Los nervios VII y VIII abandonan la cavidad cerebral cerca del orificio ótico. Los nervios IX, X y XI salen por un solo orificio grande que también aloja la vena yugular. El XII pasa al exterior a través de un orificio del exoccipital.

ON AN ENDOCRANIAL CAST FROM A REPTILE,
DESMATOSUCHUS SPURENSIS, FROM THE
UPPER TRIASSIC OF WESTERN TEXAS

E. C. CASE

University of Michigan

NINE FIGURES

As has been shown by the author¹ in a preliminary paper, the remains of *Desmatosuchus* indicate a new suborder of phytosauroid reptiles. In cleaning the skull of this specimen it was found that the brain cavity was practically undistorted and that it was possible to obtain a plastic cast of the endocranium. As is well known, any endocranial cast does not reveal the true shape of the brain, and this is particularly true of the reptiles where the brain is surrounded by a mass of connective tissue or by a large space between the pia and dura mater which is crossed by fibers of connective tissue. Nevertheless, such casts give an idea of the form and relative size of brain and the location of the cranial nerves and blood-vessels. Such casts from mammalian skulls are not uncommon in some localities as the Oligocene deposits of the Big Bad Lands of South Dakota, but very few have been found from the lower vertebrates. Even skulls so well preserved that the cavity may be cleaned out and casts made are not common. Moodie² has figured such a cast from the Pennsylvanian deposits of Kansas without assigning any taxonomic position to the specimen and has briefly reviewed the literature of endocranial casts of fossil forms up to the time of his paper. To his publication the reader is referred for a historical account of the subject and a discussion of such casts as have been found or made. Moodie mentions two papers by

¹ Journal of Geology, vol. 28, no. 6, 1920, p. 524.

² Moodie, R. L., Jour. Comp. Neur., vol. 25, no. 2, 1915.

Cope³ describing such casts, one from a cotylosaurian reptile, *Diadectes*, from the Permian of Texas, and one from a phytosaur, *Belodon*, from the upper Triassic of Texas, but he neither discusses these papers nor reproduces the figures given by Cope. This is understandable, as the figures are very poor and difficult to interpret and the subject-matter is only distantly related to the material of his paper. As the casts figured and described by Cope are from forms more nearly related to the one described in this paper, the figures are reproduced in line drawings as accurately as they may be made out.

The cranial cavity of *Desmatosuchus* was completely cleared out, leaving the surface of the bone in good condition with all the pits and foramina clearly marked. As may be seen in the figures, the cast as finally secured shows the general form and proportions of the brain cavity and the positions of the main outlets. The anterior wall of the cavity was entirely cartilaginous or membranous, and as this portion was not preserved in the fossil the opening was stopped with plastic clay which is easily detected in the figures; for this reason, the form of the olfactory tract and of the pituitary body is not completely shown.

The olfactory tract was evidently large, as in most of the primitive forms, and extended well forward directly beneath the upper wall of the skull. The cerebral portion was relatively small, scarcely any swelling being revealed in this part of the cast except at the posterior end of the prosencephalic region. The anterior-lower part of this region was enclosed by the alii, orbitosphenoid bones, and the approximation of the bones of the two sides forms notches in two places which indicate the points of escape of the nerves which supplied the eye. No indication of the origin of the II, III, or IV pair of nerves is shown on the cast and no outlets except the notches mentioned.

It is impossible in the cast to distinguish between the diencephalic and the mesencephalic regions of the brain, but the area

³ Cope, E. D., Proceedings American Association Advancement of Science, Ann Arbor meeting, 1885, and Proceedings American Philosophical Society, 1886, p. 234.

Proceedings American Philosophical Society, 1887, p. 219, and American Naturalist, vol. 22, 1888, p. 914.

occupied by these two is marked by a slight but distinct depression, which is outlined by definite elevations; the posterior one amounting to a low, sharp ridge. From this depression rise the processes, above and below, which may be referred to in general terms as the epiphysis and the pituitary body.

The upper process is complex and is, perhaps, composed of two parts. Just posterior to the edge of the prosencephalic portion there are two protuberances which mark the position of a pair of deep pits in the upper wall of the brain case. In cleaning the skull it was impossible to be certain that the bottom of these cavities had been reached, but it seemed probable that it had. With the aid of a dentist's mouth-mirror and fine curved awls the pyrite filling was picked out until it seemed that the bottom had been reached, but because of the inaccessibility of the cavities and their small diameter it is possible that the cavities may have been deeper and even that they may be the entrances to foramina. Cope, in describing the endocranial casts of a phytosaur, *Belodon*, and of a cotylosaurian reptile, *Diadectes*, speaks of the 'lateral processes of the epiphysis' and in describing the skull of *Belodon* speaks of the process as lying in a "large canal which enters the posterior part of the orbit." To this canal he gave the name of the orbitopineal canal and in certain papers speaks of the orbitopineal process on the casts. The function of this canal he was unable to determine, but suggests that it carried a nerve or blood-vessel.⁴ In his earlier papers he was inclined to the belief that *Diadectes* was blind because he could find no outlet for the optic nerve and because the structure of the animal suggested that it was burrowing in habit; as the parietal foramen is exceptionally large in this form, he was inclined to believe that the orbitopineal canal might have carried a nerve from the large, probably functional, eye which occupied the parietal foramen, which in part supplied the necessary vision. It is impossible to tell whether such a canal existed in *Desmotosuchus*, but if it was present it was very small, and the author of this paper is inclined to believe

⁴Dr. R. L. Moodie, in conversation with the author, has suggested that these processes may indicate a portion of the course of the ductus endolymphaticus.

that it did not exist. Moreover, there is a decided difference in the endocranial casts in this region. In Belodon and Diadectes the processes are large and rise from the sides of the epiphysis, in Desmatosuchus they are small and are entirely anterior to the epiphysis. It is possible that if a true cast of the brain could be obtained the origin of the processes might be found to be the same in all, but as the casts were all made from empty cavities a similar origin should be apparent.

The epiphysis is very different in form. In Belodon and Diadectes there is a strong posterior process, and Cope describes the epiphysis of the former as 'subquadrate.' The orbitopineal process extends either directly outward from the side, Diadectes, or outward and forward, Belodon. In Desmatosuchus the epiphysis is erect and narrow anteroposteriorly with no posterior process. In both Belodon and Desmatosuchus the processes referred to as the epiphysis are casts of a deep pit on the under side of the skull in the exact position of the pineal foramen in other reptiles, but in neither of these is the roof perforated. In looking up this matter the author has found that much uncertainty exists as to the exact character of this process in the brain; it is known that both the epiphysis and the paraphysis may reach large size and that either one may terminate in a functional eye; at least, either one may carry organs which possess the histological structures of the retina and the crystalline lens. In some forms there has also been found a third evagination of the brain, posterior to the epiphysis, called the pineal organ, which has a similar histological structure. Wilder, in his History of the Human Body, states that it is the paraphysis which was developed in the extinct Stegocephalia and filled the parietal foramen and the epiphysis which was developed in the reptiles, birds, and mammals. On the other hand, it is known that the epiphysis is not developed in the modern alligator. The term epiphysis is used in this paper only in a general sense and without knowledge of its true nature.

On the lower side of the diencephalic region of the cast is the second process; this represents the combined infundibulum and the saccus vasculosus, or the pituitary body. Only the posterior

and lower borders are shown, for the anterior part was enclosed by the cartilaginous anterior wall of the skull which was lost in fossilization. The process extended directly downward by a narrow neck which passed through a narrow notch formed by the approximation of the alisphenoid bones at their lower borders. Its posterior face lay against the basisphenoid bone, not penetrating it, and its lower surface is an excavation on the upper surface of the posterior part of the parasphenoid. The lower part of the process was enlarged and the posterior face, at least, extended backward at a sharp angle. The lower end terminates in a bifurcate extension which is formed by the casts of the beginnings of two foramina which open outwardly and downward in an excavation on the upper surface of the parasphenoid. These foramina continue and terminate in deep grooves on the side of the basisphenoid. On the posterior face of the process are two small prominences which mark the position of two foramina on the lower face of the basisphenoid, evidently the openings for the internal carotid arteries.

The posterior part of the depressed area mentioned above must also include the mesencephalic portion of the brain, but there is nothing to mark the presence of either optic lobes or optic thalami. This does not, however, suggest either their absence or relatively small size, for if a cast were made of the brain cavity of *Sphenodon* or of an alligator no evidence of these structures would appear, though they are of large size.

Posterior to the depressed area the whole cast is curved sharply downward and then straightened out horizontally in the metencephalic region. On the lower edges of the anterior part of this region there are large prominences which mark the position of the large foramina for the passage of the V pair of nerves. There is no indication in the cast of the division of this nerve into its parts; this must have taken place external to the cranial wall. Within and a little posterior to these prominences is indicated the position of a pair of small foramina in the floor of the skull, evidently the outlets for the V pair of nerves. Posterior to the V and at about the middle of the posterior part of the cast there are a pair of processes on each side, one almost directly above the

other. The upper pair are the casts of the otic cavities and mark approximately the position of the VIII, and probably, also, the VII nerves, for these two pairs escape from the skull of the Crocodilia in almost the same place. The otic cavities were injured in fossilization both by pressure and by the crystallization of the gypsum and pyrite which filled the cavities of the skull. It is apparent that there was a thin wall between the otic cavity and the brain cavity, but this has been so injured that it is impossible to determine the original form of the otic cavity or the form and position of the semicircular canals.

Below are the large cylindrical projections which filled large foramina carrying the IX, X, and XI nerves and the jugular vein. All of these must have escaped through a common opening, as the walls of the brain are very perfect in this place and no other openings are present.

Near the posterior end of the cast are slender processes which mark the position of the XII nerves. Above these processes there are small prominences which filled pits in the inner walls of the exoccipital bones. These pits were entirely cleared, and it is certain that they were not the beginnings of foramina; their meaning is unknown.

The whole metencephalic portion of the cast is rather high and narrow. It is possible that this is due in some degree to crushing, but there is no indication of such crushing in the skull, and it is probable that it is the true form. The whole cast is very small relative to the size of the animal, and even assuming that the brain occupied the whole cavity its size would be remarkable, though after all it is not much smaller, relatively, than the brain of *Sphenodon* or of an alligator.

It is difficult to make any satisfactory comparison of this endocranial cast with the one made by Cope from the specimen of *Belodon buceros* because of the unsatisfactory nature of his figures, but some points can be made out. As can be seen by the figures in this paper, the whole shape is different, the cast from *Belodon* does not show the sharp downward curve posterior to the middle region. The cast of *Desmatosuchus* is thinner for its height and does not have so long a metencephalic portion.

The epiphysis lacks the posterior prolongation, certainly it is not 'subquadrate' in form, and the lateral processes rise in front of, not at the sides of, the epiphysis. The olfactory tracts were much broader. The optic nerves did not escape through distinct foramina. Only the origin of the pituitary body is shown in Cope's figures, but he describes it as small and occupying a fossa in the base of the cranial cavity. These characters support the evidence afforded by the bones of the skull that *Desmatosuchus* must be placed, at least, in a distinct suborder from the *Phytosauria*.

In considering the endocranial casts from the four very different primitive reptiles figured in this paper, it is certain that the brains of all had certain very distinctive characters in common. The brain cavity was relatively long and narrow with small development of the cerebral hemispheres. The optic lobes and tracts were too small to leave any distinct marks on the casts, though they were probably well developed. The brain was sharply elevated in the middle portion with large epiphysial or paraphysial processes. There was a sharp downward bend in the posterior portion. There is a considerable degree of constancy in the location of the origin of the cranial nerves. One thing is especially noticeable—the large size of the pituitary body in the giant forms. *Tyrannosaurus* was the largest of the carnivorous dinosaurs; *Diplodocus* was one of the largest creatures that has lived upon the earth; *Triceratops* was elephantine in size; *Desmatosuchus*, though not so large as the dinosaurs mentioned, was 10 or 12 feet long and probably a giant of its kind. In all of these forms the brain is exceedingly small relative to the body, but the pituitary body is very large relative to the size of the brain. The connection between the size and activity of the pituitary gland and the size and the proportions of the body in mammals is well known. Hyperpituitarism results in large size or the over-development of certain structures, as the fingers, features, etc. It is equally well known to paleontologists that one of the common variations among those which occur so abundantly in the senile stages of any phylum is giantism. The suggestion naturally rises that the disturbances which

arose in the phylum coincident with the development of adverse conditions were primarily of a physiological character affecting the deep-seated organs of the nervous system and through them, and only secondarily, the superficial structures. The teratological affections of the pituitary body which produced abnormal structures in a normal phylum may have gradually become a fixed character and resulted in normal giantism in certain groups. Similar correlations between other glands of the body and such characters as spines, horns, tusks, etc., which finally developed into excessive overgrowths in the senile stages of various phyla may have arisen in the same way.

Since this manuscript was prepared two excellent figures of endocranial casts have been published: Osborn and Mook, *Memoirs American Museum of Natural History, New Series*, volume 3, part 3, plate LXIV, Endocranial cast of *Camarasaurus supremus*; Lambe, Canada Department of Mines, *Memoir 120*, fig. 27, Endocranial cast of *Edmontosaurus*.

PLATES

PLATE 1

EXPLANATION OF FIGURES

- 1a Lateral view of endocranial cast of *Belodon buceros*. After Cope.
1b Upper view of the same. Both figures $\times \frac{1}{4}$.

ABBREVIATIONS

<i>Ep.</i> , epiphysis	<i>Olf.</i> , olfactory tract
<i>Orb.Pin.</i> , orbitopineal process	<i>Cb.</i> , cerebellum
<i>Cer.</i> , cerebrum	<i>II, V</i> , cranial nerves

- 2a Upper view of endocranial cast of *Diadectes phaseolinus*. After Cope
2b Lateral view of the same. Both figures $\times \frac{1}{4}$.

ABBREVIATIONS

<i>Olf.</i> , olfactory tract	<i>Cb.</i> , cerebellum
<i>Cer.</i> , cerebrum	<i>V</i> , cranial nerve
<i>Ep.</i> , epiphysis	<i>Ot.</i> , otic tract

- 3a Lateral view of endocranial cast of *Tyrannosaurus rex*. After Osborn.
3b Upper view of same. Both figures $\times \frac{1}{4}$.

ABBREVIATIONS

<i>Olf.</i> , olfactory tract	<i>I to XII</i> , cranial nerves
<i>Cer.</i> , cerebrum	<i>h1</i> and <i>h2</i> , epiphysis
<i>Opl.</i> , optic lobe	

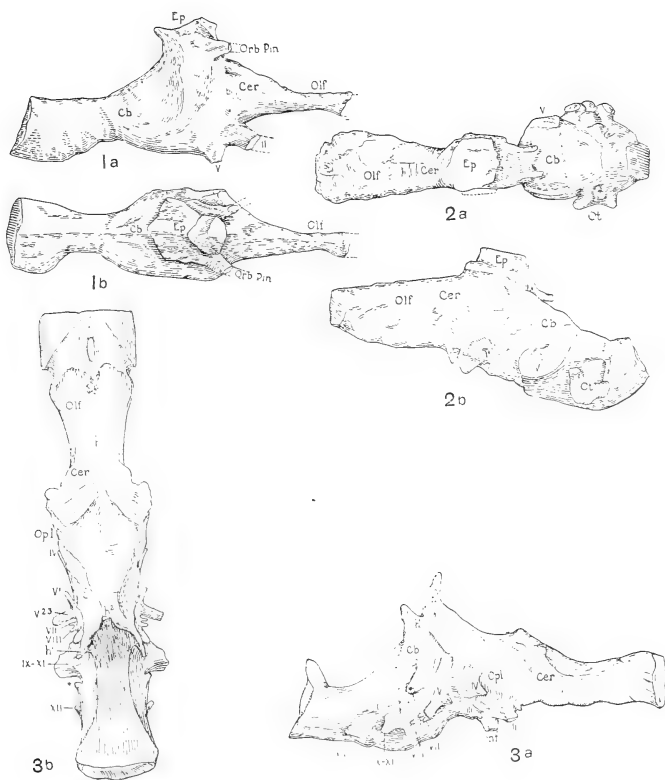


PLATE 2

EXPLANATION OF PLATES

$\frac{1}{4}$ Lateral view of endocranial cast of *Diplodocus longus*. After Osborn.
 $\times \frac{1}{4}$.

ABBREVIATIONS

Sac.End., saccus endolymphaticus *II to XII*, cranial nerves
Int.Car., internal carotid arteries

5a Upper view of endocranial cast of *Triceratops serratus*. After Hatcher from Marsh.

5b Lower view of same. Both figures $\times \frac{1}{4}$.

ABBREVIATIONS

Olf., olfactory tract *Ve.*, vein
Cer., cerebrum *Pit.*, hypophysis
Cb., cerebellum *II to XII*, cranial nerves

6a Lower view of endocranial cast of *Triceratops serratus*. After Hay. This and the two following figures give Hay's interpretation of the casts, as opposed to that given by Hatcher.

6b Upper view of same.

6c Lateral view of same. All figures $\times \frac{1}{4}$.

ABBREVIATIONS

Olf., olfactory tract *Car.*, carotid artery
Cer., cerebrum *Ju.v.*, jugular vein
Ve., vein *II to XII*, cranial nerves
Pit., hypophysis *Ep.*, epiphysis
Op.a., base supposed ophthalmic artery

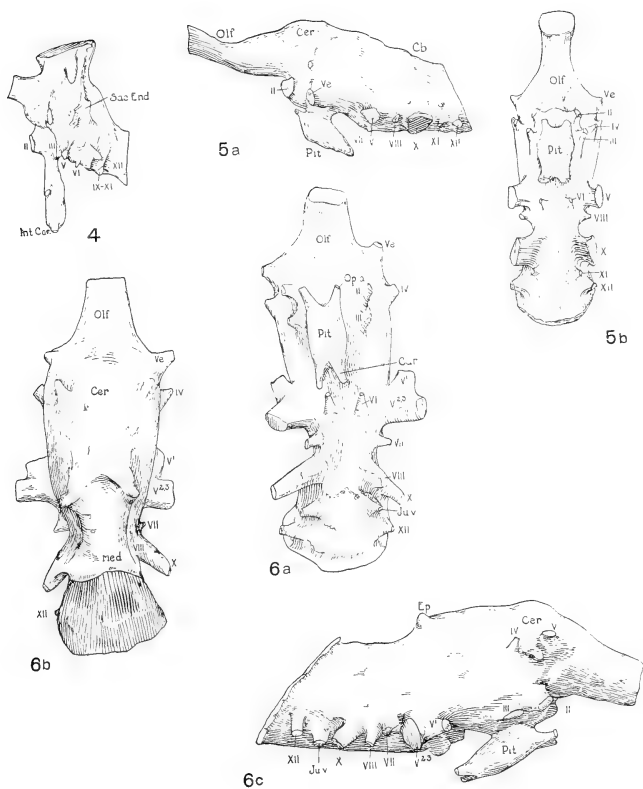


PLATE 3

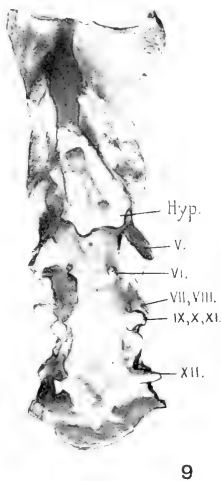
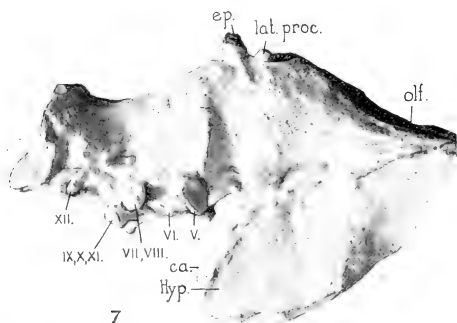
EXPLANATION OF PLATES

- 7 Lateral view of endocranial cast of *Dematosuchus spurensis*.
- 8 Upper view of same.
- 9 Lower view of same. All figures $\times \frac{1}{2}$.

ABBREVIATIONS

Ep., epiphysis
Lat.proc., lateral processes
Olf., olfactory tract

Ca., carotid artery
Hyp., hypophysis
II to XII, cranial nerves



Resumen por el autor, J. M. D. Olmsted,
University of Toronto.

Los efectos de la resección del nervio lingual del perro.

Los botones gustativos desaparecen de las papilas fungiformes en la parte anterior de la lengua del perro como resultado de la resección del nervio lingual. Solamente desaparecen los botones gustativos del lado de la lengua innervado por el nervio que se ha cortado, mientras que los del otro lado persisten sin cambio alguno. Este resultado se debe a la resección del nervio y no a otros trastornos producidos por la operación. El proceso que sigue a la sección de dicho nervio es un proceso de degeneración acompañada de la acción fagocítica de los leucocitos, no un proceso de desdiferenciación o metamorfosis. El punto que ocupaban los botones gustativos viene a ser reemplazado por células epiteliales.

Translation by José F. Nonidez
Cornell Medical College, New York

EFFECTS OF CUTTING THE LINGUAL NERVE OF THE DOG

J. M. D. OLMSTED

Department of Physiology, University of Toronto

SIX FIGURES

It has been found that cutting the glossopharyngeal nerve of the dog results in the disappearance of the taste buds in the circumvallate papillae on the side of the tongue innervated by the severed nerve (Vintsehgau und Höningschmied, '76; Vintsehgau, '80; Drasch, '87; Sandmeyer, '95; Semi Meyer, '96). Those investigators who studied the manner of disappearance of the taste buds, Vintsehgau and Meyer, were convinced that degeneration did not take place, but rather a process of dedifferentiation or metamorphosis transposed the sense cells into indifferent epithelial cells. Ranvier ('88) performed this same operation on the rabbit and claimed that the sense cells did undergo degeneration and were completely destroyed on the spot. In another paper (Olmsted, '20) I have described the changes occurring in the taste buds situated on the barbels of the fish, *Amiurus nebulosus*, after cutting the appropriate branches of the facial nerve. With the fish, as with the rabbit, there were distinct degenerative processes observable at a certain time after the operation, accompanied by phagocytic action of leucocytes. I suggested that similar degenerative changes would probably be observable in the dog if one could happen upon a preparation taken at just the correct interval after severing the nerve.

The mammalian tongue is innervated by two nerves, the glossopharyngeal, supplying the posterior third (including the circumvallate papillae), and the lingual, supplying the anterior two-thirds. All the operations on mammals to which reference has been made were on the glossopharyngeal nerve. This paper

gives the results of severing the lingual nerve and shows the correctness of the supposition that the taste buds would disappear through a process of degeneration—not dedifferentiation or metamorphosis.

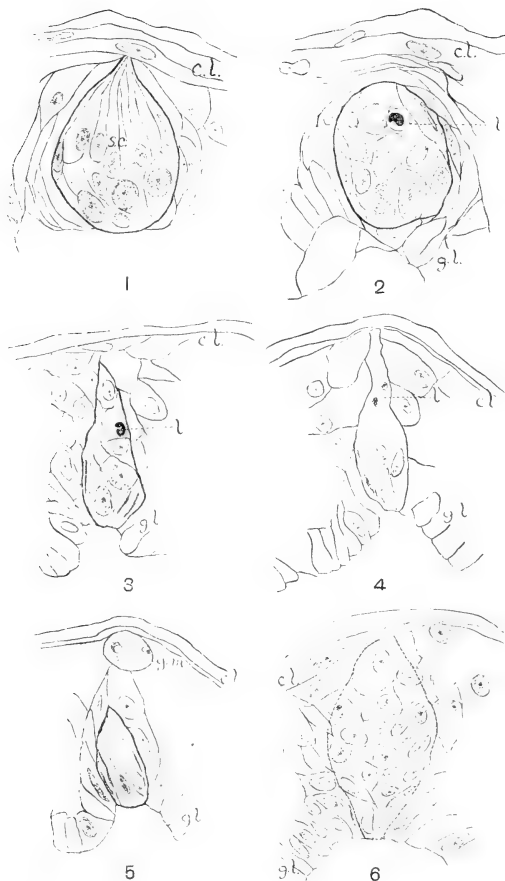
The dogs were anesthetized, and after shaving the region under the jaw, a slit was made through the skin at the inner border of the jaw, usually on the right side. The thin mylohyoid muscle was cut through and the fascia separated so as to disclose the loop of the lingual nerve just peripheral to the branch to the submaxillary gland. This can be done with practically no interference to the blood supply of the tongue. Only a small amount of bleeding takes place in the skin. A small piece of the nerve, about 5 mm. in length, was excised. The cut edges of the mylohyoid muscle and the fascia were sewn together with sterile gut, and some six stitches were sufficient to close the skin over the wound. Often the dogs began to eat within six hours after the operation and none of them seemed seriously inconvenienced, being able to move their tongues in a fairly normal fashion. There was never any infection or supuration. Swelling did occur, but this disappeared before the end of a week.

When sufficient time had elapsed after an operation the dog was killed and the tongue removed. The taste buds on the anterior part of the tongue are situated on the fungiform papillae. These papillae occur singly and at irregular intervals over the surface of the tongue, being more numerous at its tip. With a little care they can be recognized, especially with the aid of a hand lens, and removed from the tongue together with a few of the encircling filiform papillae. Ten or more fungiform papillae were taken at random from the dorsum and sides of the anterior half of the tongue on the side corresponding to the severed nerve, and also for control a similar number from the unoperated side. These excised papillae were fixed in Zenker's fluid containing acetic acid, in formol-Zenker, or Heidenhain's osmic-acetic-sublimite mixture (Heidenhain, '14). Sections were cut 8 μ in thickness. The usual haematoxylin stains were used.

No pronounced changes in the taste buds from the operated side could be detected after twenty-four hours, nor even after three days, except perhaps in the latter case there might be a diminution in size of the taste bud, as if the cells were not so well filled out as normally. In one dog which was killed the eighth day after operation there were found eighteen fungiform papillae without a taste bud and with no particular arrangement of the epithelial cells to even suggest where taste buds had been; three papillae each with the remains of one taste bud, as in figure 4; and two papillae each with one perfect taste bud, and one of them having also the remains of a second. On the unoperated side ten fungiform papillae were examined. Each of them had two to five taste buds in perfect order, with an average of three to a papilla. When these were compared with taste buds from the tongues of normal (unoperated) dogs no differences could be detected. Forty fungiform papillae from unoperated dogs were examined, and with one possible exception none contained less than two taste buds, and there was an average of four to each. This possible exception was a very small papilla resembling a filiform papilla in the thickness of its cornified layer, but in its shape a fungiform papilla. This particular papilla was the only one of the forty which possessed no taste buds.

In one very large dog the following record was obtained fifteen days after operation. On the operated side there were six fungiform papillae without sign of a taste bud; three with the arrangement of epithelial cells showing the former position of taste buds, and two having the remains of one taste bud and also a single perfect one in each. On the unoperated side five papillae were examined. Each had taste buds in perfect order with an average of five to a papilla. Two other dogs gave similar results.

To test whether the disappearance of the taste buds was due to the injury incident on the operation, one dog was treated exactly as the others, i.e., the lingual nerve was disclosed, etc., but the branch to the submaxillary gland was cut between the lingual nerve and the salivary gland, and the lingual left intact. Eleven days after the operation, seven fungiform papillae from



All the figures were drawn under a camera lucida at a magnification of 1500 and reduced one-half in reproduction. The boundary of each taste bud is marked by a heavy line. *c.l.*, cornified layer of epidermis; *g.l.*, germinative layer of epidermis; *g.m.*, granular mass of extruded cytoplasmic debris; *l.*, leucocyte; *s.c.*, sense cells.

the operated side were examined, and each possessed taste buds in good order, having an average of four to a papilla.

The process by which the taste buds disappear is shown in the figures. One finds in many of the taste buds on the tongues of unoperated dogs leucocytes which resemble the large lymphocytes of the blood. In a very few cases I observed polymorphonuclear leucocytes within the taste bud, as in figure 2. The first portion of the taste bud to disintegrate is the region just above the nuclei of the sense cells (figs. 3, 4). This is exactly similar to what I found in the fish. Furthermore, the debris seems also to be extruded through the pore by which the taste bud communicates with the surface, for, as figure 5 illustrates, there was found at the pore of one taste bud a mass of granular material, evidently cytoplasmic, since it stained faintly with eosin, exactly similar to that inside the taste bud. Within this mass were two nuclei, one evidently belonging to a leucocyte. In the dog whose taste buds were examined on the eighth day after operation there were many cases of mitosis observed, in one instance three in a single field. In the forty fungiform papillae from normal dogs there was not a single case of mitosis, nor were there any in the neighboring filiform papillae in the operated dogs. The final process consists in filling up the site of taste buds with epithelial cells (fig. 6).

In a few cases where degeneration had evidently occurred recently, the boundary of the taste buds was still fairly evident in the form of elongated cells, as contrasted with the more or

Fig. 1 Longitudinal section through normal taste bud from fungiform papilla of dog.

Fig. 2 Tangential section through normal taste bud, showing presence of leucocyte.

Fig. 3 Longitudinal section through taste bud of dog whose lingual nerve had been cut eight days before. The area surrounding the leucocyte is entirely empty. The whole bud is considerably shrunken.

Fig. 4 Longitudinal section similar to figure 3. Distal portion of taste bud empty except for a leucocyte and a disintegrating nucleus. The more proximal part is filled with granular material.

Fig. 5 Tangential section through same taste bud as figure 4. Mass of granular material lying at the neck of the pore of the taste bud.

Fig. 6 Fifteen days after operation. Taste bud indicated by arrangement of cells. Possible boundary indicated by broken line.

or less hexagonal cells inclosed by them. In other cases there were no evidences whatever of taste buds. In all respects is this similar to what was found in *Amiurus*.

SUMMARY

1. Taste buds disappear from the fungiform papillae on the anterior part of the dog's tongue as a result of cutting the lingual nerve.

2. Taste buds disappear only on the side of the tongue corresponding to the severed nerve, those on the other side remaining in perfect order.

3. The process by which the taste buds disappear is one of degeneration with the aid of phagocytic leucocytes, and not one of dedifferentiation or metamorphosis.

4. Epithelial cells take the place of the former taste buds and during this process there is marked proliferation by mitosis from the germinative layer.

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Resumen por el autor, Howard Ayers,
Cincinnati, Ohio.

Los nervios espinales ventrales del *Amphioxus*.

Los nervios espinales ventrales del *Amphioxus* son nervios delgados y largos, y no estructuras cortas en forma de pincel o ramillete, extendiéndose a lo largo de todo el miotomo. Cuando los fascículos terminales más pequeños abandonan el nervio, se curvan fuertemente para entrar entre las placas musculares y se separan sobre la superficie de las fibras musculares formando las últimas fibrillas nerviosas que penetran dentro del músculo. Los nervios espinales dorsales emiten ramas que entran en los espacios inter-miotómicos y envían finos nervios dentro del cuerpo de los miotomas, probablemente de naturaleza sensorial. La semejanza de los nervios espinales ventrales del *Amphioxus* con los nervios ventrales motores de las formas más superiores es, pues, bien manifiesta.

Translation by José F. Nonidez
Cornell Medical College, New York

VENTRAL SPINAL NERVES IN AMPHIOXUS

HOWARD AYERS

SEVEN FIGURES

The spinal cord of *Amphioxus* presents a sinuous outline, when viewed either from the dorsal or ventral face. This sinuosity is due to the curved projections of the cord (fig. 6) where the motor roots issue, and since they emerge, as a rule, alternately on the right and left sides, the superficial curvatures result. The main structures of the cord are not affected thereby.

The motor roots are composed of fibers which lack protective covering of any kind. The number of fibers varies from about 100 to 250, in keeping with the variation in the size of the myotomes. The larger the myotome the greater the number of muscular elements requiring nerve control. The terminal myotomes in head and tail are the smallest of the lot and the size increases in both directions toward the middle of the body. The motor nerves increase in size in the same way—the increase in size being due solely to the addition of nerve fibers.

It has been stated that no motor nerve has been found for the first myotome. I have dissected the first myotome and find the nerve supplying it small, and could locate with certainty only two branches, one for each fork of the myotome.

Rhode's description of the motor nerve is more complete and accurate than his figures, but is far from correct either as to the size and the course of the nerve or its method of branching. It is, however, the best I have seen.

He describes three sections of the motor nerve, an anterior which spreads like a fan on the dorsal part of the myotome, a median section composed of ventral fibers which run to the rectus abdominis and the ventral portion of the outer part of the myotome, and a posterior section or bundle of fibers which

passes in between the rectus abdominis and the longus dorsi and supplies both these muscles.

The current opinion seems to be that "the motor nerves spread out like a fan and terminate upon the muscle fibers of the myotomes," to use Willey's phrasing. This is not correct, for the ventral spinal nerves of *Amphioxus* are relatively long nerves and the muscle fibrils most distant from the cord are controlled by means of nerve fibers which span the interval between the spinal cord and muscle. The longest of these fibers are, consequently, at least as long as the vertical diameter of the animal, since the fibers run ventrocaudad in the ventral fork of the myotome.

Another point should be kept in mind—the motor root divides into nerve branches, distinct and compact bundles which, while without noticeable sheaths, nevertheless hold their fibers firmly together as nerves, to give them off as they pass along the territory of their distribution by the familiar method of nerve branching until the ultimate nerve filaments are set free singly or in small groups of two to eight fibrils. These single fibers or small groups are the elements which pass to the muscle for contact or fusion and also for the formation of nerve nets upon the surface of the muscle plates.

It is common experience of those who have tried to lay bare the connection of the ventral spinal nerves with the cord, that the root too easily tears away from the cord at its junction therewith. A dissection of a motor nerve with the parts in place is shown in figure 1. The fibers issue from the ventrolateral angle of the cord as a wide, thin plate of fibers, which, as Rhode has shown, is composed of groups of bundles of fibers. This differentiation in the structure of the root I have followed back into the spinal cord. It indicates a specialization of the nerve supply assembled from several parts of the cord for the innervation of the structures under the control of the ventral roots.

The motor roots have been thought to belong exclusively to the striated muscles of the myotomes but, as indicated in figures 1, 2, and 3, there is a small bundle of fibers (relatively too large in the figures) marked 5, which runs out with the

large bundle 3, but which does not enter the myotome, passing along its inner face to join the nerve plexus of the aortic trunk. It is a splanchnic nerve. All the other branches of the ventral nerve, so far as I have observed, are distributed to the myotome.

The four branches to the myotome are, counting from before backward: nerve 1 leaves the anterior part of the root and runs dorsolateral and enters the dorsal fork of the preceding myotome; nerve 2 is the main supply of the dorsal fork of the myotome belonging to the root, it runs between the two sections of the muscle and extends the entire length of the muscle; nerve 3, the largest and longest given off by the ventral root, enters the posterior edge of the ventral fork of the myotome belonging to the root, between the closely applied surfaces of the longus dorsi and the rectus divisions of the muscle ventrad of the junction of the two forks and runs the entire length of the ventral fork; nerve 4 runs caudad and enters the tip of the ventral fork of the next succeeding myotome; while nerve 5, as stated above, runs into the body to join the nerve plexus associated with the central vascular mechanism. The ventral spinal nerves are therefore composite nerves supplying not only the trunk muscles, but also visceral organs.

In life, the muscle plates are semifluid and are applied close to the connective structures forming the walls of the base of the dorsal fin, the spinal cord, the notochord and the sheet that forms the supporting wall of the body cavity. The trunk muscle is attached to this connective-tissue skeleton and some of the fibers in the vicinity of the motor nerve interdigitate among the fibers of the issuing ventral root.

The fact that muscle fibers are attached to the dural sheath and penetrate among the bundles of nerve fibers of the ventral root was largely responsible for the controversy as to whether the motor fibers are striated like the muscle fibers. None of the nerve fibers show striation. Rhode's figure 34 shows two bundles of muscle fibers which he calls striated nerve fibers. These belong to the group of muscle fibers indicated in figure 4, *M''*, some of which attach direct to the dura.



ABBREVIATIONS

A-B line indicates the position of the aorta with reference to the myotomes
C, spinal cord
D, dorsal spinal nerve
L, subdural lymph space
M, body of the myotome adjacent to the ventral root
M', muscle fibers inserting close upon the motor root
M'', muscle fibers inserting on the dura and among the fibers of the ventral root

V, ventral spinal nerve
d, ramus dorsalis of dorsal spinal nerve
1, first branch of motor root, runs to preceding myotome
2, second branch of motor root, runs to dorsal horn of myotome
3, third branch of motor root, runs to ventral horn of myotome
4, fourth branch of motor root, runs to succeeding myotome
5, fifth branch of motor root, runs to aortic plexus

The neuromuscular mechanism thus arranged insures coördination of the contraction of the myotomes of one side as a physiological unit. Besides the innervation by the motor root there is a further innervation by the branches of the dorsal spinal nerve through the ramus ventralis. These branches pass into the myotome where the visceral ramus of the dorsal nerve crosses the interspaces between the myotomes (fig. 7) and enters the muscle. These nerves probably terminate in the connective tissue, but I have not seen their endings.

Another point of interest in connection with the dorsal roots is the group of branches given off between the so-called dorsal branch and the point where they pass lateral of the myotomes (fig. 7). These branches I have not followed to their endings; they are mainly distributed to the connective-tissue structures between and about the mesial surfaces of the myotomes.

Here is material out of which the dorsal nerves of the higher vertebrates could be made up. By a reduction of the branches outside the myotomes accompanied by an increase in these mesial branches, the characteristic relation of the dorsal root in

Fig. 1 Dissection showing spinal cord of *Amphioxus* with dorsal and ventral roots and the branching of the motor nerve. The cord is represented as transparent to show the sweep of the motor fibers as they assemble to leave the ventral angle of the cord. The horns of the myotome are placed so as to show the motor nerve to advantage.

Fig. 2 Dissection of myotome of *Amphioxus* seen from mesial face to show motor nerve and its branches 1 to 5. The rectus (mesial) portion of the myotome is removed. The drawings fail to reproduce the translucent, silver-gray color of the nerves and the delicate outlines of the thin plates before they break up into the smaller nerve bundles. The branches shown in the figure are only the main bundles which lie between the lateral and mesial halves of the myotome. The innumerable branches given off from these to pass immediately among the muscle plates to their motor endings are not shown.

Fig. 3 Dissection of motor roots of *Amphioxus*, showing three adjacent nerve roots and their course in the muscles. The myotomes and ventral roots are separated longitudinally, hence the figure shows the nerves connecting adjacent myotomes as stretched to the extent of the separation of the myotomes. The double line A-B, indicates the position of the aorta in this region, to which branches of the motor roots make their way. The arrow points cephalad. The heavy lines represent the motor roots as they issue from the dura. They are displaced to the right, their normal position is along the line of junction of the ventral and dorsal forks of the myotome.

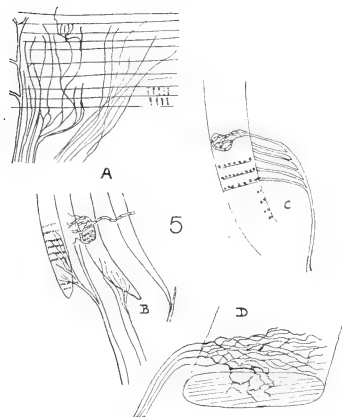
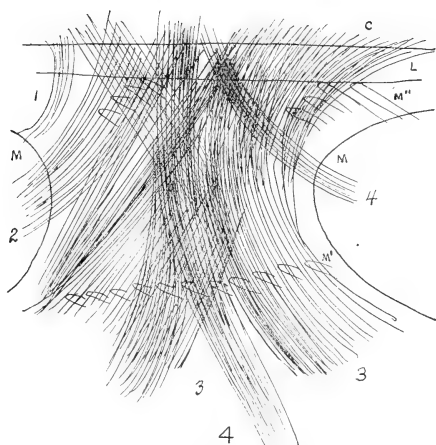
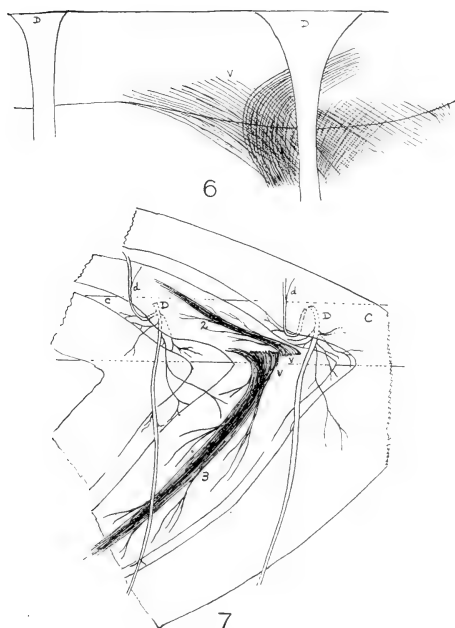


Fig. 4 Dissection of motor root of *Amphioxus* to show the motor fibers leaving the spinal cord, *C*, to pass out to their endings. *M*, group of muscle plates; *M'*, muscle elements inserting among the nerve fibers; *M''*, muscle elements inserting on and near the dura.

Fig. 5 Four illustrations of the motor nerve terminals in *Amphioxus*. *A* shows a group of fine terminal fibers crossing a muscle plate; striation of muscle



fibrils indicated. A four-branched end-plate is shown on one fibril. B shows approach and terminal branching of motor nerves on ends of muscle fibers, also one end-plate of nerve with lateral approach. C, muscle fibers more highly magnified, showing motor end-plate formed by one branch of motor nerve which broke up into six terminal fibrils. D, nerve net formed by terminal fibrils of motor nerve on surface of muscle plate.

Fig. 6 Dorsal view of dissection of spinal cord of *Amphioxus*, showing two dorsal and one ventral root. Of special interest is the dorsal root which issues from the cord, dorsal to the ventral root and on the same side of the cord. The bulbous swelling of the cord at the place of issue of the ventral root is shown.

Fig. 7 Dissection of three myotomes, two dorsal nerves and one ventral, to show relation of the latter to spinal cord and to the muscle segments. The group of branches from the dorsal nerve given off near the place of issue between the myotomes is largely submyotomic, only the distal parts arriving at the surface of the myotomes. The branches from the nerve given off where the nerve trunk crosses over the interseptal spaces are indicated; some of these go to the body of the myotome.

the higher forms would come into being. I think this transformation has taken place in the evolution of the vertebrate stock.

Regarding the termination of the fibers leaving the cord in the ventral root, it is plain that not all of them form end-plates on the muscle (fig. 5). Some of them form nerve nets which lie superficially upon the muscle plates and are apparently inter-muscular fibers. There are, however, gradations between a few anastomoses between adjacent fibers of a bundle and the extreme of complicated nerve net. In the former condition I have found anastomosing fibers to terminate in end-plates, but where an extensive net is formed I have not seen terminals leaving it to enlarge into end-plates. They may occur, nevertheless, as in a few instances fibers from the superficial net seemed to penetrate into the muscle plate (fig. 5, D). The figures of Kutchin and Dogiel drawn from methylen-blue and Golgi preparations do not show the actual structure of the end-plates.

My observations of the neuromotor mechanism of *Amphioxus* indicate that, while both nerve and muscle structure is much less differentiated than in the higher forms, it is doubtless an ancestral stage. The addition of sheaths would convert the nerve fibers into the structures we find higher up. *Bdellostoma*, *Ammocoetes* and *Petromyzon* furnish some of the intermediate stages to this process of acquisition of protective coverings by the nerve fibers. The same is true as regards the relation of the dorsal and ventral roots. *Amphioxus* has them as separate and distinct bundles throughout their root territory, but they are found ending peripherally in the same territory (the myotomes), but on different business. Again, *Bdellostoma*, *Ammocoetes*, and *Petromyzon* present us with stages which show how the condition in the higher forms has been arrived at.

The ventral root is relatively as extensive as in the higher forms, i.e., it runs to the extreme limit of the muscle organ to which it is assigned and is a long branching nerve, not a brush of fibers.

Resumen por la autora, Ada Roberta Hall,
University of Oregon.

Regeneración del cordón nervioso de un gusano anélido.

La autora ha llevado a cabo diversos experimentos sobre el cordón nervioso de la lombriz de tierra, *Helodrilus caliginosa*, mediante simple sección y también mediante resección de varios segmentos, con el fin de estudiar los tejidos comprendidos en el proceso de la regeneración, el papel de las células sanguíneas y el origen de las fibras nerviosas regeneradas. Las conclusiones de estos experimentos son las siguientes: 1) Existe una rápida formación de material de cicatrización, el cual procede de las células sanguíneas, un tejido fácilmente obtenible para llenar el espacio producido por el corte. 2) Existe un crecimiento definido de las fibras nerviosas a partir de los extremos del cordón hacia la banda formada por el tejido de cicatrización. También pueden emigrar dentro de esta banda las grandes células ganglionares, si el corte se ha practicado a través de un ganglio, reemplazando de este modo el tejido en su condición normal. 3) Si se extirpan varios ganglios se forma una banda de cicatrización del mismo modo que en la regeneración normal, para llenar el espacio producido por el corte, y las fibras y células ganglionares son reemplazadas por el crecimiento de los extremos cortados. Después de dejar pasar un largo periodo (dos a tres meses) para que continúe el crecimiento después de haber vuelto a presentarse los movimientos normales, todas las células superfluas son reabsorbidas de tal modo que el gusano aparece completamente normal. Aunque el tiempo necesario para la regeneración parece notablemente rápido, las medidas tomadas por la autora tienden a indicar que cuando se comparan con la regeneración de las fibras de los vertebrados, este tiempo tan corto en el anélido se debe a la escasa longitud de la fibra más bien que a su crecimiento más rápido.

REGENERATION IN THE ANNELID NERVE CORD

ADA R. HALL

Zoological Laboratory of the University of Oregon

SEVENTEEN FIGURES (SIX PLATES)

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INTRODUCTION

The earliest work on regeneration in the earthworm is concerned with the general growth of an individual or with the grafting together of parts of the same or different species of worms. Heschler ('98) and Rand ('08) have outlined the steps in the process when a new head is formed, four or five segments having been removed. They consider that the ectoderm is responsible, at least in part, for the growth of the new nerve material. Friedlander has shown that the pull of the longitudinal muscles will cause coordinated rhythmical movements in two pieces of worm when their only connection with each other is a piece of string. Biedermann shows that the nerve cord is important in that it may carry impulses through several

segments when the muscles are dead and are fixed to prevent 'pull.' Bovard ('17) says that not only does the nerve cord carry impulses through several ganglia, but that muscle pull reinforces these impulses in each segment so that the message goes a long distance without losing its force. He has also in his histological studies shown that when the nerve cord is cut regeneration is very rapid, and that the fibers which are regenerated bear a very direct relation to the movements of the worm, the locomotor movements depending on the fine central fibers, which regenerate first, and the general collapsing movements depending on the giant fibers which recover about twenty-four hours later. My problem has been to discover in the process of regeneration, a single cut having been made: 1) what cells form the cicatrix region and unite the ends of the nerve cord and, 2) whether nerve fibers actually grow through this region from the ends of the nerve cord before impulses can pass from one end of the worm to the other, or whether the muscle layers serve as a bridge to get the impulses past the gap. In connection with this, I have also studied different periods of regeneration in worms from which two or three segments of cord have been removed, in order to see if this process will throw any light on simple regeneration.

DISCUSSION OF THE PROBLEM

A. Materials and methods

The worm used in these experiments is *Helodrilus caliginosa*, commonly found in our fields and meadows, especially where there is turf. They are kept several days between moist cloths to free them from as much grit and dirt as possible. Cloths are preferable to filter-paper, as they can be sterilized frequently and they also prevent the filling of the digestive tract with paper pulp—a hindrance in sectioning. When ready to operate, the worms are anesthetized by placing them in 5 per cent alcohol until movements practically cease; left longer they are not strong enough to recover and may die before regeneration is complete. The worm is next laid over a rounded surface, such

as a cork, under a binocular microscope. A small obliquely transverse slit is cut in the epidermis, a few metameres posterior to the clitellum. Then with a needle sharpened to a knife edge on one side, the nerve cord is picked up and cut. Care must be taken not to sever the ventral blood-vessel, as too severe bleeding is injurious. The worm is then replaced in the moist cloths.

When removing several segments of the cord, a transverse slit is cut in the ventral epidermis as before, and then the skin is carefully lifted up with the point of the scissors and cut longitudinally for several segments. When placed under the binocular, the nerve is seen lying in the furrow made by the cut. The sharpened needle is inserted and the cord cut. One can then count down as many segments as he wishes, insert the needle and cut again. The section of cord is removed with the forceps and dropped into alcohol, which causes the ganglia to form contraction knots. These may be counted as a check on the number of segments removed. The skin wound usually closes in from two to four hours. The jars and cloths in which the worms are kept are thoroughly cleaned with hot water each day to prevent infection.

B. Technique

In order to study the process of regeneration, a large number of worms were operated on and then killed at intervals of fifteen minutes for the first six and a half hours. This series was stained by the Hardesty method no. 1, which gives good fiber differentiation and is supposed to show mitosis, if any be present. Erythrosin and toluidin blue were the stains used. Although cellular and intercellular structures are clearly contrasted by this method, the killing agent used is a poor one where delicate scar tissue is involved, as the violent action of the absolute alcohol tends to pull the cicatrix loose from the wound on one or both sides. Another series gives fifteen-minute intervals of regeneration, up to an hour and ten minutes, then for periods of several hours up to four days. This series was killed in Perenyi's fluid and stained with iron haematoxylin and orange G.

For a study of the time of regeneration ordinary haematoxylin and eosin give very good slides. For a further study of cell and fibrillar structure, different staining methods were tried. Picrosulphuric acid or Perenyi's fluid for killing agent, with iron haematoxylin and orange G, gives good cell and fiber differentiation. The slides should be very heavily stained and then differentiated slowly for the best results. Another method which gives good fiber stain is Lewis' pyroligneous acid method. Tissues are killed in von Rath mixture for eight days. They are then washed in methyl alcohol, placed in 50 per cent pyroligneous acid solution for forty-eight hours, then washed in absolute alcohol for several days. Paraffin sections were cut 10μ thick.

Several silver-nitrate methods were tried, but with little success. It was thought that the intravital methylene-blue method would give clear fiber areas. This method was tried repeatedly, but the penetrability of the substance is poor where the central nerve cord and its sheath are concerned. Even when injected into the coelom and left for varying lengths of time, there seemed to be no reaction to it in the cord.

For comparative study smears were also made. These were of three kinds: 1) pure blood from the ventral vessel at the anterior end; 2) body fluid; 3) cicatrix fluid drawn from a cut which has been open a few seconds. To get only pure blood it was necessary to make a fine capillary pipette, lift the vessel on the forceps away from all other fluids, and press the pipette into it. The contraction of the vessel will force the blood up in the tube rhythmically. The other fluids were easily obtained. These smears were dried; some were stained by Wright's method for blood corpuscles, and others directly with erythrosin and toluidin blue or Hardesty's method no. 1. Films were also made and allowed to stand for half an hour in a moist place in order that the corpuscles might expand. These were then killed in corrosive-acetic fixing fluid and stained with eosin and methylene blue.

C. Regeneration after a simple cut

1. *The source of the cicatrix cells.* In studying the source of the plug cells formed when a cut is made in the body wall, all of the tissues of the normal worm should be considered (fig. 1) and comparison made with these same tissues in the regenerating worms.

The epithelium of the normal worm is of the simple columnar type. Its appearance varies somewhat according to its position on the metamere. On the outer rounded parts of the metamere is a high columnar epithelium with many gland cells, large rounded bodies closely packed with granules. In the furrows are the low columnar type with few or no gland cells. When a cut is made in the body wall the epithelium is one of the first tissues to regenerate. Worms killed within a few minutes after section show the edges of the epithelium turned inward (fig. 2). This is due to the contraction of the circular muscles. In a very short time—forty-five minutes to two and a quarter hours—the wound is filled with a mass of cicatrix cells which extends up, nearly to the digestive tract and fills all the space between the cut ends of the different layers (fig. 3). The inturned edges of the epithelium may be imbedded in this mass, but the epithelial cells which are covered thus break down and degenerate without leaning toward or migrating into the plug. Figure 13 shows such a condition (*D. Ep.*). The cells at the outer edge lean out, and by proliferation give rise to the new epithelium (*R. Ep.*). More often the skin is not turned in so deeply and the growth is from the cut end of the epithelial layer (fig. 14).

An early stage in the process is shown in figure 12. Here the plug (*Pl.*) fills the cut area, with a few strands of circular muscle imbedded in it. The new low epithelial cells extend out a short distance, but the central part of the plug is directly exposed. As regeneration progresses the whole plug shrinks and the epithelial cells grow entirely across (figs. 14 and 15). Rand ('01) has shown that these arise from the old epithelium and migrate across the cicatricial area. Although the old cells tend to lean in the direction of growth, yet there seems to be no proliferation

of these cells inward through the plug toward the nerve cord. We may conclude therefore that the epithelium does not give rise to the cicatrix cells nor to the regenerating nerve cord, but merely covers over the exposed area formed by the cut.

There are two muscle layers: the circulars, lying just beneath the epithelium, forming a band around each metamere (fig. 1, *C. M.*), and the longitudinals, which extend lengthwise, forming a muscular cylinder just inside the circulars (*L. M.*). As stated before, the first reaction of the muscles when a cut is made is the contraction of the circulars. This tends to draw the cut edges of epithelium together and also holds the longitudinal muscle ends toward each other. The formation of the plug is very rapid; at first it merely fills the spaces, showing no direct connection with the muscles. It is easily seen that these cells are not proliferations of the muscle layers. As regeneration progresses, the plug cells elongate and orient themselves with their long axis in the direction of the muscle pull.

In figure 15 longitudinal fibers are seen between the cut ends of the longitudinal muscles (*L. M. F.*), and between these and the epithelium is an area of cut ends (*C. M. F.*), showing that the circulars are also connected by these fibers. Friedlander suggests that the plug cells come from the muscles, and from the shape of the nuclei after a day or two of regeneration one might be led to think so. However, from the rapidity of formation and the appearance of the cells in the earliest stages this is not probable. At a very early stage (two and a half hours, fig. 3), the plug is formed and closely packed with round nuclei, but there is no particular direction to the fibers. At ten hours (fig. 12) the nuclei are still large and round. Only in the later stages do the nuclei elongate and the fibers show a definite orientation. I believe that this is a mechanical effect, in part at least, due to the pull of the old muscles after a definite amalgamation has taken place between their raw edges and the cells of the plug. Figure 6 shows the close relationship between the plug and the muscle fibers.

Heschler believes that the subsequent growth and regeneration comes as a proliferation of the old muscle cells or of undif-

ferentiated cells scattered through these layers, but since the plug cells are mesodermal in origin, as I shall show later, and are capable of changing shape under the influence of a steady pull exerted along one axis, it seems probable that these plug cells might also develop contractile fibrils and fill the gap caused by the cut. More work is needed to determine whether this latter step actually takes place or not. The early stages of plug formation and cell elongation and orientation in response to muscle pull, are clearly shown in many of my slides.

The digestive tract lies as a smaller tube within the cylindrical body wall. As a rule, it is not cut during an operation and does not enter into very close relationship with the plug cells. Occasionally small fragments of chlorogogue cells may be distinguished, by their granular nature, in the plug, but their position here is accidental rather than purposeful.

Between the digestive tube and body wall lie two other structures, the nerve cord and blood-vessels (fig. 1, *N. C.* and *Sub.*). When the nerve is first cut the elasticity of its substance causes the ends to draw as far apart as the mesenteries will permit, sometimes even slipping through the sleeve in the septum into the next segment (fig. 3). This causes the mesenteries to assume a slanting position. In addition to the small vessels of the skin and muscles, the subneural vessel is cut and there may even be breaks in the ventral vessel. This allows blood to escape into the segment where the cut is. Experiments have been made with this blood to determine the part which it may play in the regeneration process. When a cut is made in the body wall, very little bleeding occurs, except from the large vessels. The small vessels are apparently closed by the first drop of blood which comes from the cut end. The closure of even the largest vessels, dorsal and ventral longitudinal vessels, is very rapid. We may say, then, that very little bleeding occurs when an earthworm is cut.

If the ventral vessel be raised and a clean smooth capillary pipette inserted, a large amount of blood may be drawn without any closure of the cut. If this is run out under a cover-glass on a clean slide, the cover being slightly raised by a paraffin V open

at the point, the drop remains liquid for a long time. A fiber inserted at the point will cause a current in the drop and the action of the cells may be studied. There are a large number of cells in earthworm blood which seem to have an amoeboid action and may be likened to the leucocytes of higher forms. These cells, when contracted, are fairly regular, but long delicate arms of cytoplasm may be thrown out. These cells tend to aggregate in the liquid around any dust particles or fibers which may be on the slide. As other corpuscles come along in the stream and strike these clumps they also cling fast. Drew ('10), in his study of the mollusc blood (*Cardium norvegicum*), finds that agglutination takes place there also. He has caused these amoeboid cells to agglutinate on cotton fibers. As the cells strike each other, protoplasmic strands are formed between them, and each cell may then attach to a different fiber. Gradually shrinkage takes place and the fibers are drawn together. The cells of the earthworm blood agglutinate around dust particles or fibers in a drop or with each other in smears, since friction seems to be the causative factor in this process. Figure 7D shows such an agglutination taken from a smear. Figure 7B shows a contracted corpuscle with eosinophile granules; C is such a cell in the expanded condition. There are also present in the smears, cells whose cytoplasm shows basophile granules (fig. 7A).

The question then arises as to just what takes place in the blood stream and wound area when a cut is made in the body wall. Blood may flow on to a glass slide from a clean, smooth pipette without forming a compact plug, but if the same blood be poured from a blood-vessel into a cut in the body wall, a plug is formed which prevents further flow. We know that mammalian blood is caused to clot by the action of the thrombokinase in the cut tissues. If clam blood be brought in contact with friction surfaces or cut tissues, its corpuscles agglutinate. Drew believes that not only does friction of the cut surface cause this, but that some substance formed by the injury hastens the process. The earthworm blood seems to be very like the mollusc blood in its power of agglutination, so that in all probability plug

formation is due to similar conditions. The mollusc plug is due to the thick mass of agglutinated cells holding back the further flow of liquid, rather than to the formation of a true clot.

The plug which forms at a cut in the earthworm may be thought of, then, as a large mass of agglutinated cells, filling all the wound region and attached to all raw surfaces through this same power of agglutination. The subsequent shrinkage of the cytoplasm brings the nuclei closer together, giving the plug a denser appearance and also draws the cut edges toward each other through shortening of the cytoplasmic strands.

A comparative study of cells from the cicatrix of different stages shows some very interesting results. The cells from an early cicatrix (fig. 9, forty-five minutes' regeneration) show a marked similarity to the agglutinated cells of the blood smear, both in size and staining reactions. With two days' regeneration (fig. 10) the cells are smaller and more compact, while the cytoplasm shows definite fiber formation. With further growth, the cells show still more condensation, and the fibers become orientated in the direction of pull of the muscle layers and nerve cord. A comparison of figures 12, 13, 14, and 15 shows this continuous growth of cicatrix cells and fibers and their relations to other layers of the body. Figure 12, of ten hours' growth, shows large round nuclei. Figure 13, a day and six hours' regeneration, shows a condensation, with definite fiber growth and orientation. At one day and twenty-one hours the cells are still smaller (fig. 14), while at three and a half days (average time for recovery of normal movements after section of the cord) the fiber growth and orientation show a distinction in form for the two muscle layers (fig. 15, *L. M. F.* and *C. M. F.*).

A study of blood smears and films and a comparison of the cells found there with the cells of the cicatrix at different stages of regeneration lead to the conclusion that the plug is a mass of agglutinated blood corpuscles whose cytoplasm forms definite fiber connections with all raw surfaces and, through subsequent shrinkage and orientation, like tissues become united and functional in a very short period of time.

2. *Growth of nerve fibers.* The next point to be considered is the further growth in connection with the nerve cord. Does the cicatrix strand, mesodermal in origin, differentiate into nerve material, thus bringing about the early functioning of that organ, or is there a growth of nerve fibers from the cord itself? This mesodermal strand does form a bridge between the cut ends of the cord which rapidly shortens through shrinkage, but it seems more probable that the original nerve should furnish the material for nerve connections. In a study of the cord we find confirmatory evidence for this. The first step in the process is seen in figure 4, two and a quarter hours' regeneration. Here the cord has been cut between ganglia and is now united by a definite strand. The cells of the ganglia at either side have the appearance of leaning toward the cut region and the fibers are thinner at each end of the cord where the strand attaches, as if they were stretching also to fill the gap. This region is characterized by few nuclei. The central part of the strand, however, shows a large number of round nuclei, indicating a comparatively small amount of fibers. This is the region of the strand formed by the agglutinated blood-cells, while the clearer, non-nucleated strand region is formed by fibers from the nerve cord. As regeneration progresses the strand shortens through shrinkage, and these fiber areas extend farther and farther across this bridge. Now the nuclei, of necessity, appear fewer, since the fiber growths have usurped their place. In figure 5 this further fiber growth is seen, causing the nerve part of the strand to appear lighter in color than the muscle part. The fibers in the cord also show varicosities which are characteristic of nerve but not of muscle. When the cut is through a ganglion the large ganglion cells move out into the cicatrix at the same time as the fiber growth takes place, thus building up again the ganglion which was destroyed. This process shows even more clearly in the study of the regeneration, when several segments are removed. This will be discussed more fully later. When the normal functions have been recovered, the fibers extend clear across through the central region and a different more hollow part appears dorsally connecting the ends of the giant fiber (fig. 11).

The regeneration in the nerve cord consists, then, of two steps, first, the formation of a strand of plug cells which unites the ends of the cord and, second, a growth of fibers from the original cord through the strand uniting the cells of the two ganglia which were separated by the cut.

3. *Summary of simple regeneration.* We may then summarize the steps in the process when a simple cut is made in the body wall of the earthworm and the nerve cord sectioned.

1. In a very short time (forty-five minutes to two and a quarter hours) the wound is closed. This is accomplished by means of a plug which fills all the spaces between the cut ends of the tissues. The meshes of the plug are filled with corpuscles and large clumps of agglutinated cells.

2. In this same short period of time a strand of the cicatrix material unites the ends of the nerve cord, which may be as much as the width of two metameres apart, due to the elasticity of its fibers.

3. Shrinkage of the cells of the strand draws the ends of the cord nearer and nearer together.

4. A growth of characteristic fibers takes place from both ends of the cord as regeneration progresses, finally bridging the gap. If the cut be through a ganglion, large nerve cells may migrate into the connecting material reconstructing the destroyed ganglion.

5. In the final stages the different layers are connected and functioning, the muscle layers by a strand of the differentiated plug cells, and the nerve stumps by a growth of fibers from the old cord.

D. Regeneration when two to four segments are removed

1. *Former work.* In connection with my former study of the time required for a simple cut to regenerate, I operated on several worms, taking out from two to four segments of the cord. Some very striking results were apparent, but it was impossible to verify these on account of lack of material. At that time I allowed regeneration to progress to the point at which loco-

motor movements were recovered. This required fourteen days. The worm which regenerated for eleven days shows a very curious manner of growth. A strand of cicatrix cells has united the cut ends of the longitudinal muscles. This strand shows the first steps in the regeneration of that muscle layer. But further, these same cells have entirely filled the space up to the nerve cord, have become attached to its cut ends, and have drawn together across the three-metamere gap. Thus the nerve cord is united and lies on a definite strand of cicatrix tissue. The circular and part of the longitudinal muscles around the cut have been massed together between the strand and the epithelium. The digestive tract also is folded and the mesenteries of these three segments are pulled together so that they lie at an angle. This would seem to indicate that the first step in the regeneration process is the uniting of the cut ends by scar tissues.

In the worm which regenerated for fourteen days this drawing together was not so pronounced, a longer stretch of strand lying between the cut ends of the cord. The mesenteries were washed away in staining the slide, so that proof of the length of the cord was lacking. However, there was some bunching of the muscle fibers between the cord and epithelium and the cord appeared shorter than normal.

2. *Present experiments.* In my present work I have attempted to verify these results, and also have allowed worms to live for several months in dirt after recovery from such an operation, in order to see if complete regeneration of all parts takes place. Before placing them in the dirt the exact position of the cicatrix is established by counting the segments from head to cut.

In these experiments worms were killed at different intervals to get an idea of the steps in the process. Recovery of the movements occurs in from eleven to fourteen days in most cases. One worm killed after fourteen days had not recovered, but shows some interesting facts. The whole ventral part of the worm for five segments was filled in with cicatrix cells (fig. 16). These show differentiation for the muscle layers, which is apparent in the orientation of the cell nuclei. A delicate epithelium covers the lower border. Contrary to expectation, however,

there is no bunching apparent. The strand (*S*) is nearly as long as the part of the cord removed and seems to have taken its place. The septa (*Mes.*) are also practically in their proper positions, the middle segment of the three removed being shorter than normal. In the strand (*S*) of the cicatrix lying between the ends of the cord there are fewer nuclei and also there is a distinct difference in the staining reaction, this part being lighter than those each side of it. A closer study reveals the fact that it is the fibrillar structures which stain differently, those of the strand being more of a yellow with fine varicosities, while those of the surrounding cicatrix are brown. Comparison of the fibrillar structure of the strand and of the ends of the cord which we know to be nerve fibers shows that color and structure are the same, which seems to indicate a growth of nerve fibers.

After three months' growth others of these worms were killed and sectioned (fig. 17). The pieces are about ten to twelve segments long, the cicatrix being at the center of the piece. Such worms show no sign of scar in epithelium, muscle, or nerve tissue. The septa are all nearly parallel and the coelom free from extra cell structures. The new ganglia are normal in every respect except length, two of the regenerated ganglia being shorter than normal. I know that two and three segments were removed, from actually counting the ganglia on the bit of cord excised. I also know, by counting back, that the part of the worm which was sectioned contains the excised region of the cord. Thus it seems that actual growth of nerve fibers takes place, and that all superfluous cells are again absorbed, leaving all the structures normal. Since the epithelium does not grow inward when the body wall is cut, but merely covers the plug, these replaced ganglion cells as well as fibers must come from growth in the cord.

3. *Summary of regeneration when two to four segments are removed.* 1. There is a massing of cicatrix cells into all parts of the cut region as in simple regeneration. These connect all cut layers and either draw the ends of the cord directly together by means of a strand so that all the mesenteries and muscle layers become massed or the strand fills in the vacant place in the cord leaving the other layers in their normal positions.

2. Differentiation of this plug material takes place. The nuclei elongate and orient themselves in the direction of the muscle pull. Definite fibers with a dark-staining reaction are formed here also. In the region of the nerve strand the nuclei are fewer and the fibrillar material has a different staining reaction—one which is similar to that of the fibrillar portion of the original cord. This may occur as early as fourteen days and is present before recovery of movements takes place, indicating the actual presence of nerve fibers before functioning can take place.

3. Complete recovery takes place, with ganglion cells formed in each metamere of the completed cord and with the coelom entirely free from extra cell structures.

GENERAL CONCLUSIONS

1. There is a rapid formation of cicatrix material which closes the wound. This comes from the cells in the blood, a tissue easily brought in to fill the gap.

2. There is a definite growth of nerve fibers from the ends of the cord into the strand formed by the cicatrix material. Large ganglion cells may also migrate into the strand if the cut is through a ganglion, thus restoring the tissue to its normal condition.

3. If several ganglia are removed, a strand is formed as in simple regeneration to fill the gap, and fibers and ganglion cells are replaced by outgrowth from the cut ends. If a long period—two to three months—is allowed for further growth after the normal movements return, all extra cells are absorbed, so that the worm appears perfectly normal in every respect.

DISCUSSION OF RESULTS

This growth and regeneration of the annelid nerve cord seem at first glance remarkable for the speed of recovery. The cord is cut and in from four to six days the normal movements and impulses are again present in the worm. From a study of the process we may conclude that the central nervous system has

regenerated nerve fibers which connect the ganglia in front of, and behind the cut, so that normal impulses may pass. Experiments and observations on the higher forms or in man show that when a nerve is cut recovery of the lost function is very slow. Does the annelid tissue have a more rapid rate of growth than mammalian tissue? I believe not. In the earthworm the ganglia lie close together, 0.4 mm. to 0.45 mm. apart. The nerve cell processes extend from one ganglion to the next. When these fibers are cut the axon to be regenerated is very short, therefore recovery is rapid. In regenerating a simple cut, the fibers must grow from one ganglion to the next—a distance of 0.4 mm. or less. This takes four days, as a general rule, making a rate of growth about 0.1 mm. a day. In the nerve regeneration of higher forms the part cut is usually a peripheral nerve which may have its ending several feet from the nerve cell, consequently growth from the cut to the ending takes a long time. Regeneration has not been successfully brought about in the central nervous system, presumably on account of the lack of neurilemma sheaths, which, in the case of the peripheral nerve, furnish a directing path. In the earthworm the plug, with its fibrous connection, forms a path for the developing fibers through practically the entire distance traveled.

Ranson ('10), working on peripheral regeneration in the sciatic nerve of the dog, has carefully outlined the steps in degeneration and regeneration of the nerve fibers. He reports a growth of fibers for a distance of 4 mm. up the nerve and 13 mm. down from the cut during a period of twenty-five days. He has not worked out the length of time necessary for completion of the process and the return of function. This shows a rate of growth of 0.65 mm. a day. If such a growth of the peripheral fibers of the dog can be compared to the growth of the central nervous system of the earthworm, there is here a slower rate of growth for the invertebrate than for the vertebrate.

Thus, although the process of regeneration in the annelid appears at first to be remarkably rapid, the early recovery is due to the short length of fiber replaced rather than to a more rapid rate of growth.

This work was undertaken at the suggestion of Dr. J. F. Bovard at the zoological laboratory of the University of Oregon. I wish to express my appreciation to him for his kindly interest and many helpful suggestions both in the experimental work and in the revision of this paper. I am also indebted to Dr. C. H. Edmondson for the assistance and inspiration which he has given me.

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PLATES

ABBREVIATIONS

<i>B.M.</i> , basement membrane	<i>Mes.</i> , mesenteries
<i>C.M.</i> , circular muscles	<i>N.C.</i> , nerve cord
<i>C.M.F.</i> , plug cells oriented to circular muscle pull	<i>Pl.</i> , plug cells
<i>Cix.</i> , cicatrix	<i>Pl.F.</i> , plug fibers
<i>D.Ep.</i> , degenerating epithelium	<i>R.C.M.</i> , regenerated circular muscles
<i>Ep.</i> , epithelium	<i>R.L.M.</i> , regenerated longitudinal muscles
<i>Gang.</i> , ganglion cells	<i>R.Ep.</i> , regenerated epithelium
<i>G.C.</i> , gland cells	<i>Sh.</i> , sheath of the cord
<i>G.F.</i> , giant fibers	<i>S.</i> , strand
<i>Int.</i> , intestine	<i>Sub.</i> , subneural vessel
<i>L.F.</i> , central longitudinal nerve fibers	<i>Vent.</i> , ventral vessel
<i>L.M.</i> , longitudinal muscles	<i>G.Nep.</i> , glandular part of the nephridium
<i>L.M.F.</i> , plug cells oriented to longitudinal muscle pull	<i>S.Nep.</i> , saecular part of the nephridium

PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section of normal earthworm, showing relations of epithelium, muscle layers, nerve, digestive tract, and blood-vessels. Heidenhain's haematoxylin and eosin. $\times 107$.

2 Longitudinal section of earthworm killed immediately after section of the nerve cord, to show the relation of the cut ends of the cord before the regeneration process starts. Iron haematoxylin and orange G. $\times 107$.

3 Longitudinal section of earthworm killed thirty minutes after section of nerve, to show early formation of plug and its attachment to the ends of the cord. Iron haematoxylin and orange G. $\times 107$.

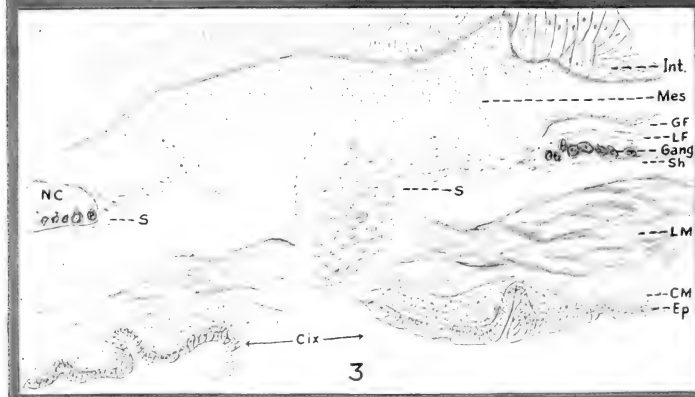
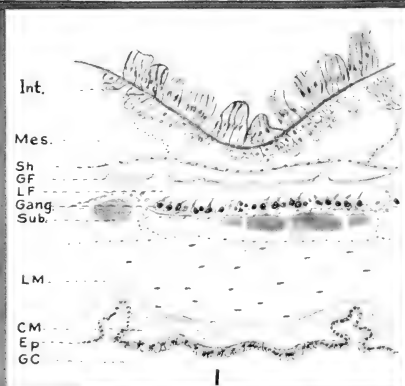
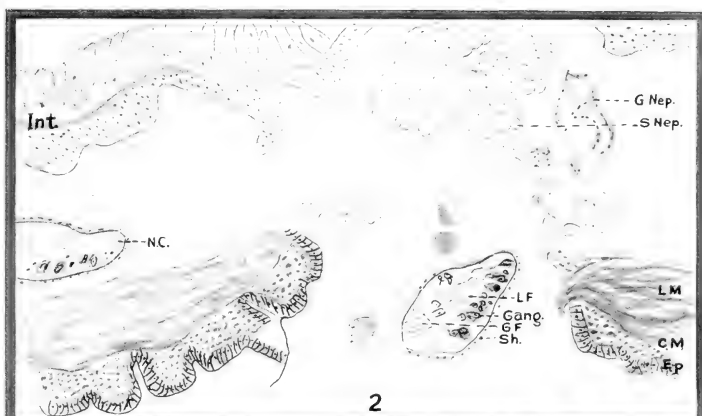


PLATE 2

EXPLANATION OF FIGURES

4 Longitudinal section of earthworm killed two hours and fifteen minutes after section of the nerve. The plug has formed a definite strand between the ends of the cord, and the beginnings of the migration and growth of nerve elements from the ends of the cord are apparent. Preserved by the Hardesty method no. 1. $\times 107$.

5 Longitudinal section of earthworm killed one day and twenty-one hours after section of the nerve. This shows growth of the plug in relation to the various layers of the body. The wound is entirely covered by the epithelium. Iron haematoxylin and orange G. $\times 107$.

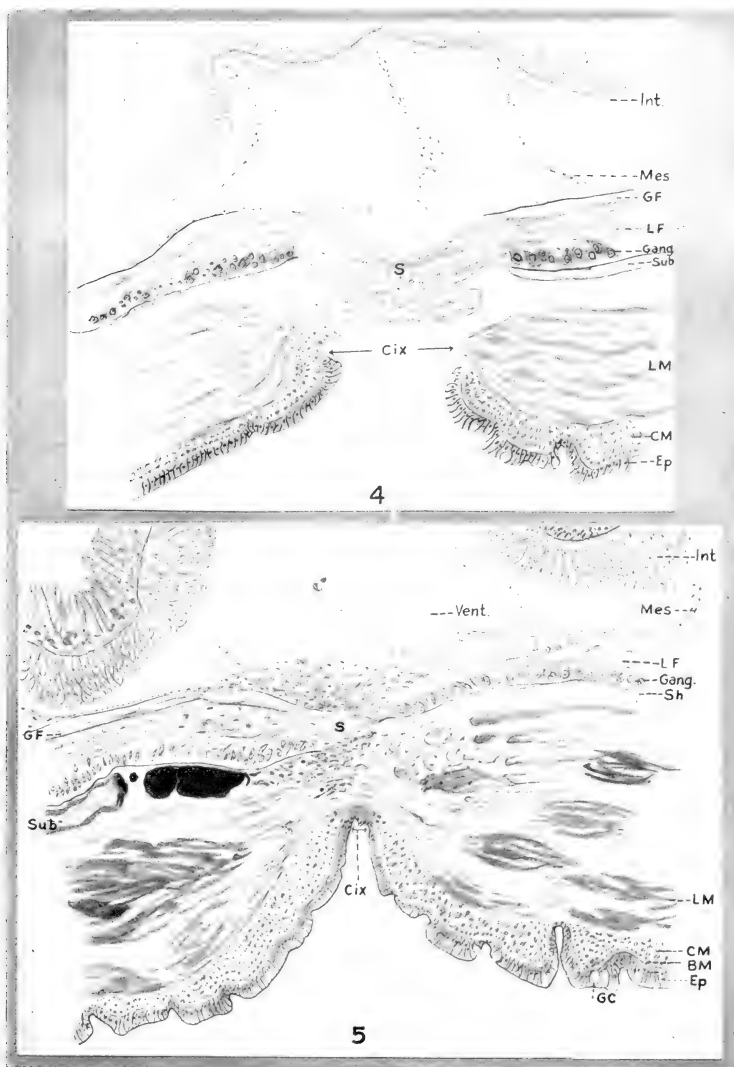


PLATE 3

EXPLANATION OF FIGURES

6 Detail of a cross-section of earthworm, regeneration complete, showing the epithelial growth and the intimate relations of the fibers of the plug to the muscle layers. Iron haematoxylin and orange G. $\times 750$.

7 Camera-lucida drawings of cells from blood smears and films, stained with Wright's stain for blood corpuscles. $\times 1700$. A. Contracted corpuscle with basophile granules. B. Contracted corpuscles with eosinophile granules. C. Same as B showing cytoplasm expanded. D. Clump of agglutinated corpuscles.

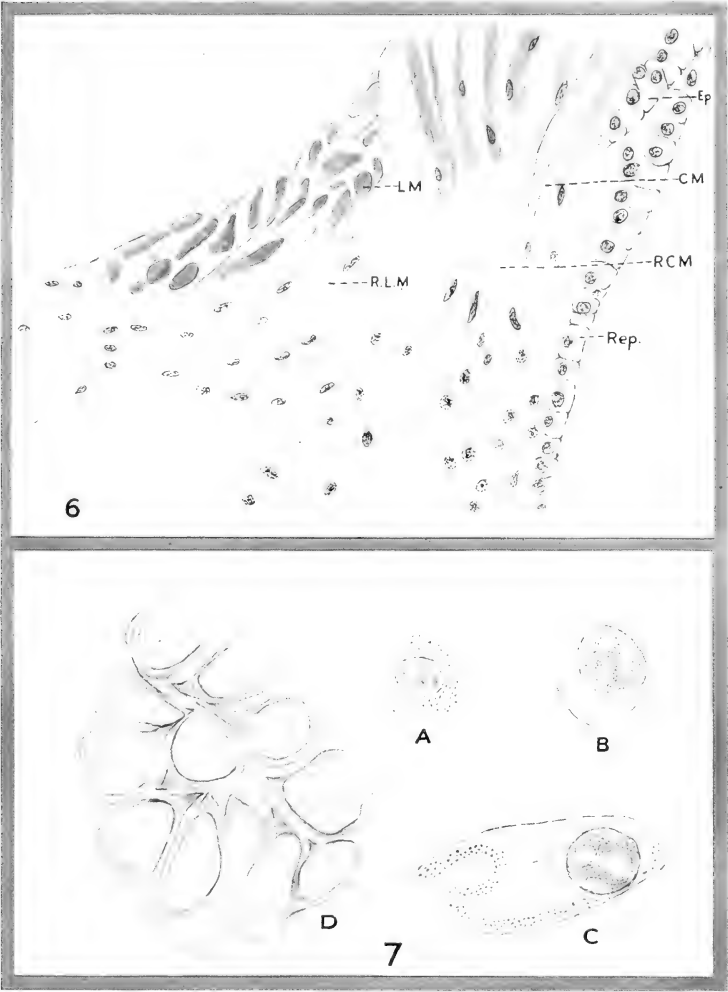


PLATE 4

EXPLANATION OF FIGURES

8 Clump of agglutinated blood corpuscles taken from a smear stained with erythrosin and toluidin blue. $\times 1700$.

9 Camera-lucida drawing of plug cells from the cicatrix of a worm killed 45 minutes after section of the cord. Stained with Hardesty's method no. 1. $\times 1700$.

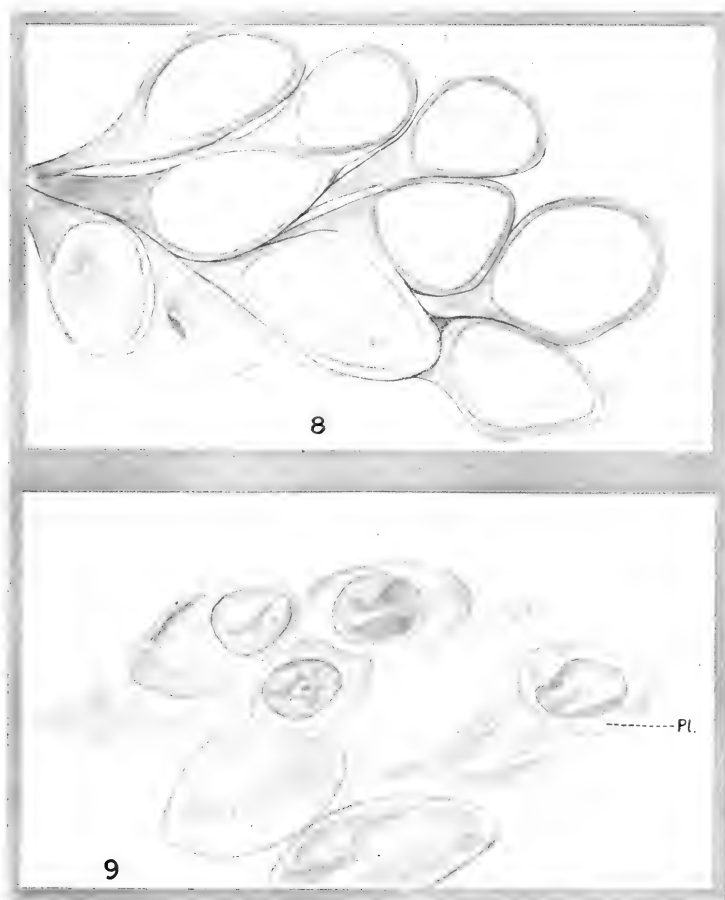


PLATE 5

EXPLANATION OF FIGURES

10 Camera-lucida drawing of plug cells from the cicatrix of a worm killed two days after section of the nerve. Stained with iron haematoxylin and orange G. $\times 1700$.

11 Detail of the nerve cord of a regenerated worm; time, four days. This shows the position of ganglion cells and the characteristic varicosities on the fibers. Iron haematoxylin and orange G. $\times 170$.

12 A portion of the body wall was removed, 0.5 mm. wide and three metameres long, in order to watch regeneration in the muscle layers and epithelium. Worm killed after ten hours' regeneration, showing the size and character of the plug cells and the epithelial growth. $\times 480$.

13 Same as figure 14. Worm killed after one day and six hours' regeneration.

14 Cicatrix region after one day and twenty-one hours' regeneration. This worm had only a simple cut in the body wall. $\times 480$.

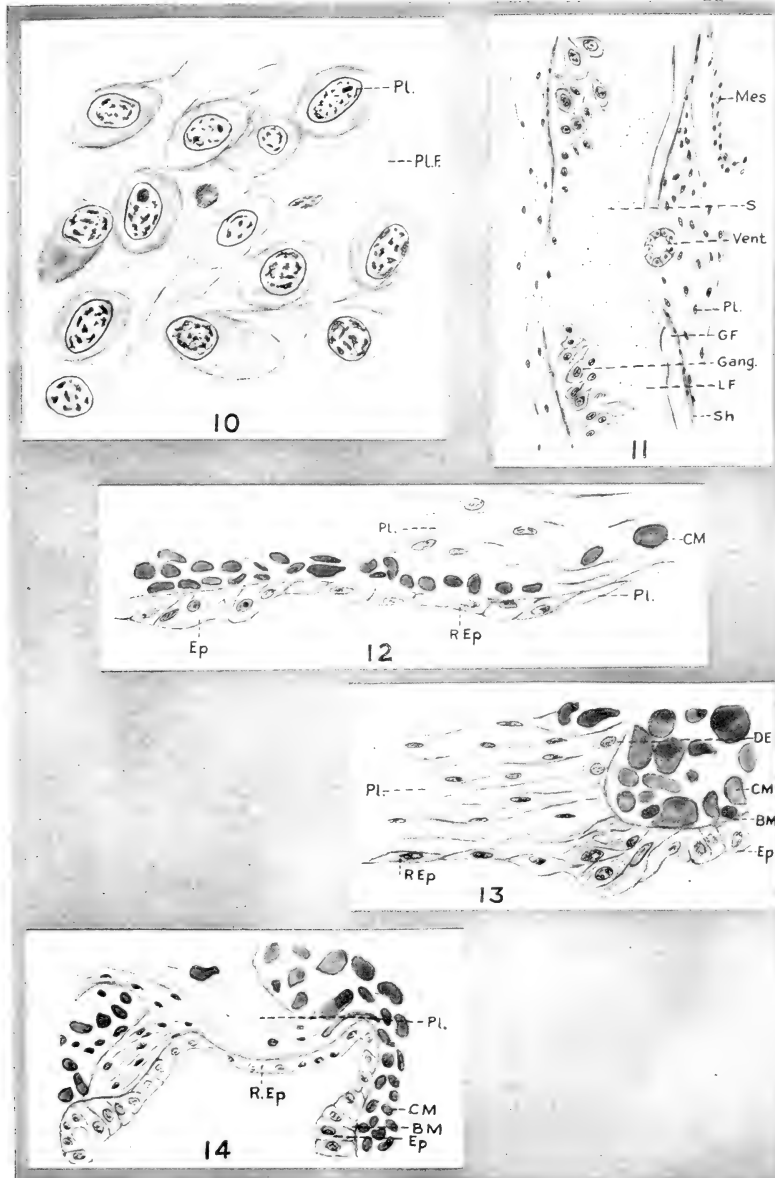


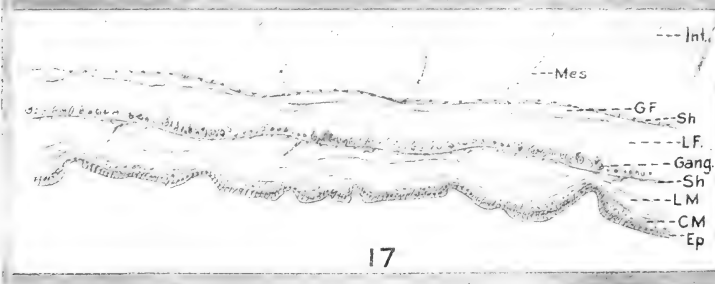
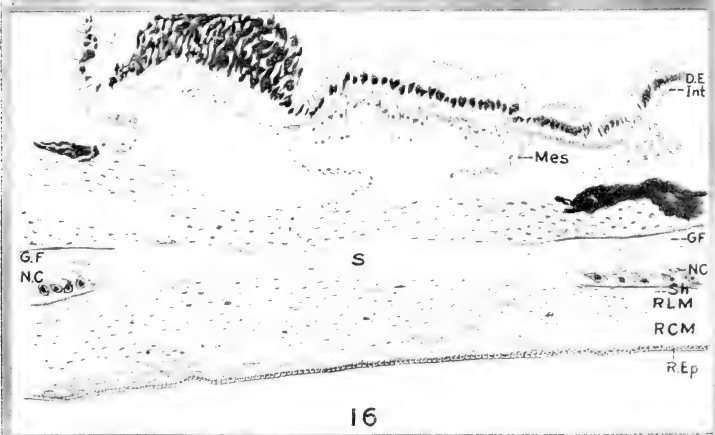
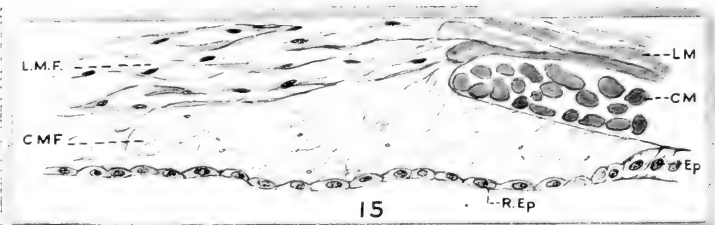
PLATE 6

EXPLANATION OF FIGURES

15 Same as figure 14. Worm killed after three days and twelve hours' regeneration.

16 Worm killed fourteen days after removal of three segments of the cord. Regeneration not complete. $\times 107$.

17 Worm having three segments removed, grown in dirt for three months after normal movements had been recovered. All tissues appear normal except that two ganglia are shorter than the rest. $\times 107$.



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Resumen por el autor, Edward Horne Craigie.

La vascularidad de la corteza cerebral en la rata albina.

La vascularidad relativa de las cinco láminas que se distinguen en una misma región cortical es semejante en todas las áreas corticales examinadas y en todos los casos la lámina granular interna (IV) es la más rica. Las capas supragranulares presentan una tendencia a ser más ricas que las infragranulares, y la capa más pobre es la lámina multiforme (VI). Parece probable que las capas granular y supragranular sean receptivas y asociativas en función, mientras que las infragranulares dan lugar a fibras corticofugas. Esta particularidad invita a una comparación con los centros inferiores, en los cuales los núcleos sensoriales y los de correlación han sido encontrados más vascularizados que los núcleos motores. La vascularidad media de las cinco capas es la misma en las áreas occipital y temporal, y es solamente un poco menor en la región precentral. El área parietal es claramente más rica en capilares que las otras, mientras que la corteza insular es la más pobre. La vascularidad de las cinco láminas de la corteza insular es semejante en su extensión a la de los diversos centros motores estudiados en el tallo cerebral y médula espinal, mientras que la de las otras áreas corresponde aproximadamente a la de los núcleos sensoriales y correlativos. La vascularidad de la corteza cerebelar es próximamente del mismo orden de magnitud de la de la corteza cerebral. En la corteza las diferencias sexuales y raciales aparecen más marcadas que las otras partes del sistema nervioso central estudiadas previamente. El área insular parece diferir de las restantes en algunos puntos mucho más que las demás áreas entre sí.

Translation by José F. Nonidez
Cornell Medical College, New York

THE VASCULARITY OF THE CEREBRAL CORTEX OF THE ALBINO RAT

EDWARD HORNE CRAIGIE

Department of Biology, University of Toronto

FIVE FIGURES

The work upon which the present paper is based is an extension of that previously reported by the writer ('20). In the former publication an account was given of the relative vascularity of various parts of the spinal cord, brain stem, and cerebellum of the albino rat, and this account is extended in the present communication to include the cerebral cortex of the same animal. In such a connection, there comes up for consideration not only the richness of the capillary supply of the cerebral cortex as compared with that of the parts previously studied, but also the relative vascularity of the various regions into which the cortex has been divided by physiologists and histologists. Also, little, if at all, behind these problems in interest comes the question of the conditions in the various layers which characterize the cortex.

The more important accounts of the cortical localization in rodents, in so far as they are based upon histological studies, are reviewed by Sugita in his paper ('17) on the growth in thickness of the cortex. He also correlates the various regions in which his measurements were made with the detailed description by Fortuyn ('14) of the conditions in the Norway rat and with the anatomico-physiological terms of Brodmann ('09).

Sugita found the cortex of the albino rat, as Fortuyn had found that of the Norway rat, to be divisible, in typical localities, into five laminae. His description of these laminae is as follows:

The cerebral cortex of the albino rat has five cell layers if a typical locality be taken. The most external layer is the lamina zonalis (I), which has a few scattered glia-cells. Under this, there is the lamina

pyramidalis (III) consisting of typical, deeply-staining, pyramidal cells lying closely together, which corresponds to the third layer of Brodmann ('09). In the rodent brain, the lamina granularis externa (II), or second layer of Brodmann, is always indistinct, and it is almost impossible to distinguish it from the lamina pyramidalis (III). Beneath the lamina pyramidalis, the lamina granularis interna (IV) is situated, composed of crowded, deeply-staining, small granules, somewhat resembling glia-cells. Below this layer, there is the lamina ganglionaris (V), which has dispersed, large-sized, deeply-staining pyramids. Next to the lamina ganglionaris, there is the lamina multiformis (VI) with polymorphous cells.

MATERIAL AND METHODS

The material consisted of eight of the ten brains used in the previous study ('20), to which reference is made above. These were numbered 16, 23, 24, 26, 31, 55, 56, 58, for the last four of which the writer is indebted to The Wistar Institute. The forebrains of the remaining two specimens used in the earlier work were, unfortunately, not in good enough condition for study. A full account of the animals from which these brains were obtained, as well as of the technic employed in preparing them and of the method of measuring the capillaries, is given in the former paper. It will be sufficient to repeat here that nos. 23, 24, 31, and 58 were male albino rats, nos. 16, 26, 55, and 56 females. Numbers 16, 23, 24, and 26 were obtained in Toronto, while the remaining four, as mentioned above, were secured at The Wistar Institute. The microscope, lenses (Leitz no. 7 objective and no. 3 ocular), and square-ruled micrometer employed in making the measurements were the same ones as were used before, but owing to the thinness of the cortical laminae, it was found much more convenient to measure the capillaries enclosed in only one half of the large square on the micrometer. Hence the measurements made were the total length of the capillaries enclosed in an area of $\frac{1}{2} \times 189^2$ sq. μ in each section, the length of one edge of the micrometer square being 189μ . As before, such measurements were made in the same region of each of ten successive sections, and as these sections were 20μ in thickness, the sum of the (10) readings gave the total length of the capillaries in a block of tissue of the volume $\frac{1}{2} \times 189^2 \times 200$ c. μ . These results are shown in the accompanying table (table 1).

The cortical localities selected for study are those general regions in which Sugita's measurements were made. The following is a list of these localities, using Brodmann's terminology, with the number of Sugita's region to which the position of the present measurements in each corresponds approximately, and the designations of the areas used in figure 1.

BRODMANN'S TERM	SUGITA'S NUMBER	DESIGNATIONS OF AREAS IN FIGURE 1
Regio praecentralis.....	II	A
Regio occipitalis.....	IV	D
Regio parietalis.....	VII	B
Regio insularis.....	VIII	E
Regio temporalis.....	XI	C

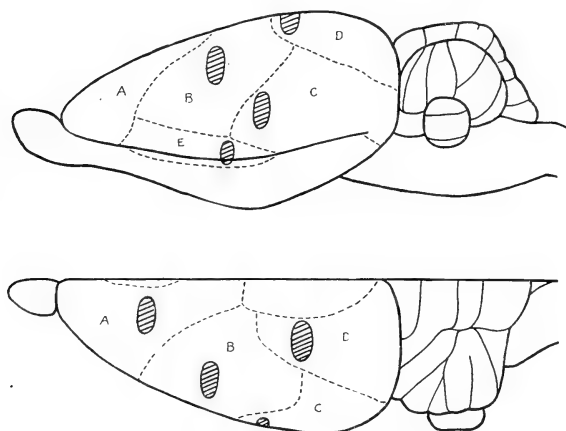


Fig. 1 Lateral and dorsal views of the left half of the brain to show the regions in which the measurements of vascularity were made. The exact location and relations of each of the areas represented is based on a comparison of figures by Fortuyn and Sugita, the names being applied in accordance with Sugita's correlation of Brodmann's terminology with these figures. Comparison with Brodmann's own figures and description seems to indicate that the anterior portion of area B is equivalent to the regio postcentralis. A, regio praecentralis; B, regio parietalis; C, regio temporalis; D, regio occipitalis; E, regio insularis.

TABLE 1
Linear measurements obtained in μ per $\frac{1}{2} \times 189^2 \times 200$ c. μ

CEREBRAL CORTEX												HORIZONTAL AVERAGES	PROBABLE ERROR OF HORIZONTAL AVERAGES
Area	Layer	F.R. 16, TO- RONT	M.R. 23, TO- RONT	M.R. 24, TO- RONT	F.R. 26, TO- RONT	M.R. 31, W. I.	F.R. 55, W. I.	F.R. 56, W. I.	M.R. 58, W. I.				
Insular.....	Lam. zonalis, I.....	2856	2203	2327	1914	2554	2620	2358	2743	2447	3.00		
	Lam. pyram., III.....	3536	2654	2496	2527	2634	2886	2304	2904	2743	3.28		
	Lam. gran. int., IV.....	3811	3086	2853	3016	3296	3135	2496	3497	3149	3.03		
	Lam. gangl., V.....	3853	2346	2604	2747	2642	2496	1898	3176	2720	4.90		
	Lam. multif., VI.....	2897	2541	2598	2922	2186	2510	1983	2855	2562	3.16		
Average.....		3391	2566	2576	2625	2662	2729	2208	3035				
Praecentral.....	Lam. zonalis, I.....	2560	2254	3387	2720	3654	3982	3723	4649	3366	5.71		
	Lam. pyram., III.....	2955	3000	4421	3910	3927	4060	3700	5073	3881	4.29		
	Lam. gran. int., IV.....	3424	4013	5082	4447	3932	4571	3813	5237	4315	3.42		
	Lam. gangl., V.....	3172	3394	4071	4105	3493	4154	2879	4134	3675	3.27		
	Lam. multif., VI.....	2803	2585	3159	3234	3010	2764	1995	3001	2819	3.34		
Average.....		2983	3049	4024	3683	3603	3906	3222	4419				
Occipital.....	Lam. zonalis, I.....	2581	3034	3424	2808	3797	4557	3531	3972	3463	4.48		
	Lam. pyram., III.....	3253	3131	3864	3893	4431	4148	3312	4719	3844	3.59		
	Lam. gran. int., IV.....	3919	3444	4485	4218	4542	4846	3708	5438	4325	3.56		
	Lam. gangl., V.....	3625	3284	4062	3892	4621	4474	3135	4496	3949	3.51		
	Lam. multif., VI.....	3364	2531	3275	2834	3972	3103	2638	3956	3209	4.17		
Average.....		3348	3085	3822	3529	4273	4226	3265	4516				

Temporal.....	Lam. zonalis, I.....	2951	2780	3582	2548	2945	4489	2986	4340	3328	5.25
	Lam. pyram., III.....	3581	3689	4290	3416	3875	5184	3525	4835	4049	3.87
	Lam. gran. int., IV.....	4332	3769	5261	4711	4528	4966	3827	4832	4528	2.79
	Lam. gangl., V.....	3217	3514	3904	3880	3525	4327	2959	3888	3652	2.85
	Lam. multif., VI.....	3135	2819	3831	3329	2805	3417	2807	3731	3234	3.05
	Average.....	3443	3314	4174	3577	3536	4477	3221	4325		
	Lam. zonalis, I.....	3056	3150	3587	3464	3468	3860	4293	4942	3728	4.01
	Lam. pyram., III.....	4288	3705	4458	4158	4959	4756	4235	5539	4512	2.98
	Lam. gran. int., IV.....	4471	4404	5156	5047	5343	5083	4931	6726	5145	3.32
	Lam. gangl., V.....	3863	3161	4115	4452	3793	3905	3214	4820	3953	3.11
Parietal.....	Lam. multif., VI.....	3854	2601	3602	3663	3359	3125	2547	3698	3306	3.64
	Average.....	3906	3464	4184	4157	4184	4146	3844	5145		

The accompanying illustrations (fig. 1) show these areas with the points where the measurements of vascularity were made indicated approximately by the hatched spaces. The outlines of the cortical regions are based upon a comparison of Sugita's reproduction of Fortuyn's figures with the former author's own illustrations of sections, and his correlation of both these with Brodmann's terminology. The outline of the brain was taken in the first place from two injected specimens which had been carried only as far as the 70 per cent alcohol stage in preparation.

The laminae of the cortex were by no means easily distinguished in many cases, the simple picric-acid stain which was employed not being well adapted for such a purpose. By careful comparison, however, with a series of sections stained with toluidine blue and erythrosin and with the specimens in which the layers were more distinct, they were located fairly accurately, it is believed, even in the more difficult cases.

Figure 2 shows the cell lamination and the vascular supply in the occipital cortex of one rat.

OBSERVATIONS

The measurements obtained are presented in table 1. The results for the eight brains have been averaged and the probable error of the averages calculated, these figures being given at the right-hand side of the table. The probability of error tends, of course, to be a little higher than was the case in the previous study on account of the use of only eight brains instead of ten. The ratios of the averages to the readings for the ventral white funiculus and the ventral gray cornu of the spinal cord in the same individuals¹ are given in the last columns of table 3. The sex of each rat has been indicated in table 1 by placing M. or F. in front of its identification number, while its locality is shown by adding 'Toronto' or 'W. I.' (The Wistar Institute).

In the preparation of the tables the mean values for the five laminae in each area, as shown in table 1, were averaged, and the areas were arranged in order of increasing vascularity, as thus determined. These averages are recorded in table 2. The

¹ See Craigie ('20).

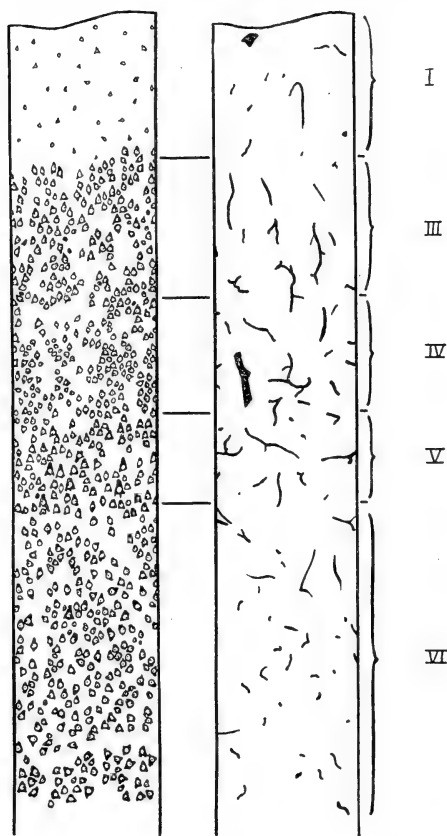


Fig. 2 On the right, a drawing of the blood vessels in a small part of the occipital cortex of R 26. On the left, a diagram of the cell lamination in the same piece of cortex. $\times 112$. I, lamina zonalis; III, lamina pyramidalis; IV, lamina granularis interna; V, lamina ganglionaris; VI, lamina multiformis.

average vascularity of the five layers is the same in the occipital and temporal regions, and it is very little less in the praecentral region. The parietal region is distinctly the richest, while the insular is much the poorest.

With regard to individual variation, it is noticeable that the same individuals tend to give rather high or rather low results in all the cortical areas, but the values obtained in the lower, subcortical centers of these individuals were not always similarly high or low. These differences are reflected in the ratios of the cortical values to those for the ventral white column and the ventral gray cornu of the cord, which have been set down in a detailed table, not published, from which table 1 has been con-

TABLE 2

Average vascularity of five areas of the cerebral cortex. (The averages of the values shown for the five laminae of each area in the second last column of table 1)

CORTICAL AREA	μ PER $\frac{1}{2} \times 189^2 \times 200$ c. μ
Insular.....	2724
Praecentral.....	3611
Occipital.....	3758
Temporal.....	3758
Parietal.....	4128

densed, and which show considerable fluctuation. The individual ratios referred to have all been averaged, and the results are found to correspond so satisfactorily with the ratios of the average readings, shown in table 3, that it is considered sufficient to publish the latter.

The averages for the various layers of each area, as recorded in next to the last column of the first table, are represented graphically in figure 3, which illustrates the relation between the different regions as well as that between the five laminae in each.

It will be observed that the relative vascularity of the five laminae in the various areas studied is fairly constant, not only as regards the averages, but even, though to a smaller extent, in the different individuals, as shown in table 1. The greatest irregularity which appears is in the case of R.56, in which the

TABLE 13
 Table showing the average vascularity of each of the parts of the central nervous system studied, expressed in terms of the length in μ of the capillaries in a cube of tissue of 100 μ edge

REGION	μ PER 100 ³ c. μ	RATIO		CEREBRAL CORTEX		μ PER 100 ³ c. μ	RATIO	
		Ventral white	Ventral gray	Area	Lamina		Ventral white	Ventral gray
Fasc. cuneatus.....	184	0.93	*		Lam. zonalis, I.....	685	3.46	0.76
Ventral column.....	198 ¹	1.00			Lam. pyram., III..	768	3.88	0.85
Lateral column.....	223	1.13			Lam. gran. int., IV	881	4.46	0.98
Pyramidal tract.....	350	1.77			Lam. gangl., V.....	761	3.85	0.85
Fasc. long. dors.....	426	2.15			Lam. multif., VI...	701	3.63	0.80
Subst. gelat., Rolandi.....	582	2.94	0.65		Lam. zonalis, I.....	912	4.76	1.05
Nuc. mot., VII.....	732	3.70	0.81		Lam. pyram., III..	1086	5.49	1.21
Nuc., XII.....	802	4.06	0.89		Lam. gran. int., IV	1208	6.11	1.34
Nuc. mot., V.....	817	4.13	0.91		Lam. gangl., V.....	1029	5.20	1.14
Ventral horn; cord.....	900 ²	4.55	1.00		Lam. multif., VI...	789	3.99	0.88
Spinal V. nucleus.....	923	4.67	1.03		Lam. zonalis, I.....	969	4.90	1.08
Deiters' nucleus.....	935	4.73	1.04		Lam. pyram., III..	1076	5.44	1.20
Mollec. layer; cerebellum.....	996	5.04	1.11		Lam. gran. int., IV	1211	6.12	1.35
Dorsal horn; cord.....	1008	5.10	1.12		Lam. gangl., V.....	1105	5.59	1.23
Inferior olive.....	1076	5.45	1.20		Lam. multif., VI...	898	4.54	1.00
Superior olive.....	1120	5.67	1.25					
Chief sens. V. nucleus.....	1130	5.71	1.26		Lam. zonalis, I.....	931	4.71	1.04
Granule layer; cerebellum.....	1227	6.20	1.36		Lam. pyram., III..	1133	5.73	1.26
Nuc. dentatus.....	1272	6.43	1.41		Lam. gran. int., IV	1268	6.41	1.41
Chief vestib. nucleus.....	1363	6.90	1.52		Lam. gangl., V.....	1022	5.17	1.14
Dors. cochlear nucleus.....	1472	7.45	1.64		Lam. multif., VI...	905	4.58	1.01
					Lam. zonalis, I.....	1013	5.28	1.15
					Lam. pyram., III..	1263	6.39	1.40
					Lam. gran. int., IV	1440	7.28	1.60
					Lam. gangl., V.....	1106	5.60	1.23
					Lam. multif., VI...	923	4.67	1.03

¹ The original average from which this figure was calculated was used as the standard for the ventral white matter.

² The original average from which this figure was calculated was used as the standard for the ventral gray matter.

lamina zonalis tends to be richer as compared with the other layers than is the case in the rest of the brains studied. This is believed to be due to an unexplained vacuolate appearance

Vascularity

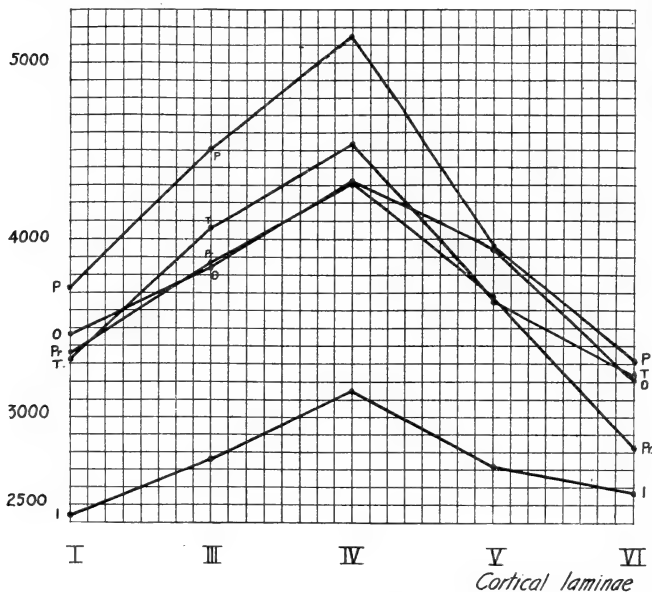


Fig. 3 Chart showing the relative vascularity of the five cortical areas studied and of the five laminae in each.

I, lamina zonalis
 III, lamina pyramidalis
 IV, lamina granularis interna
 V, lamina ganglionaris
 VI, lamina multiformis

I, insular area
 O, occipital area
 P, parietal area
 Pr, praecentral area
 T, temporal area

in this specimen of all the layers except the lamina zonalis. It seems probable that this condition of the tissue has resulted in the measurements for all the inner layers in R.56 being a little lower than they should have been. As these results fell

within the range of variation of the other individuals, however, it was decided not to discard them.

In all the five areas studied, the lamina granularis interna (IV) is decidedly the richest, the lamina pyramidalis (III) coming next, with the lamina ganglionaris (V) very little behind it. The fourth in order of richness is the lamina zonalis (I), while the lamina multiformis (VI) is the poorest in every region except the insular. It may be remarked that the lamina granularis interna, which is absent from the praecentral region of many mammals, is distinct, though thin, in that portion of the cortex of the rat, and it is interesting to note that it is not relatively poorer in its capillary supply there than in the other areas.

These observations may be compared with the description of Duret ('74) fifty years ago. He found that the outer 0.1 mm. of the human cerebral cortex contained large quadrangular meshes parallel to the surface, the next 2 mm. is filled with rather fine polygonal capillary meshes, while the inner 1 mm. has a transitional network with larger meshes, which, however, are much less elongate than those of the white matter into which they pass.

There is little certainty at the present time regarding the functions of the different layers of the cerebral cortex. Bolton ('00) noticed twenty years ago that, while the deeper layers did not vary appreciably in thickness as a result of age or chronic insanity, "there is an almost exact correspondence between the thickness of the conjoined first and second layers² of the cortex and the degree of amentia or dementia existing in the patient." Nine years later, Kappers ('09) concluded upon the basis of comparative evidence that "the granular layer in the cortex is primary in character, and has originally receptive functions," that the subgranular layers (V, VI) have chiefly the functions of projection and intraregional association, while the supragranular layers (II, III), which are the last to appear phylogenetically, are concerned chiefly with association of a higher order (inter-regional), including intellectual processes.

² The 'second layer' of Bolton appears to be equivalent to Brodmann's laminae II and III, i.e., the supragranular layers.

Brodmann (*loc. cit.*) considers Kappers' theory groundless, describing it as a wild hypothesis which is quite untenable and erroneous. Van Valkenburg ('13, '13 a [?]), however, has brought forward certain evidence which seems to support Kappers' view; as have also Nissl, Nieuwenhuijse, and Bielschowsky, whose contributions are summarized by Van't Hoog ('20). The last-mentioned author also adduces further evidence in favor of the theory of Kappers, and stresses particularly the rôle of the cells in the lamina granularis interna. These he considers, emphasizing a point concerning which Kappers had been less positive, to be 'matrix cells,' i.e., cells which have retained much of their primitive character and potentialities, which are still capable of a wide range of differentiation, and from which the other layers have probably been derived phylogenetically. It may be pointed out that, in the ontogeny of the cerebral cortex of the rat, this layer is the last one to become distinguishable according to Sugita ('17), but, on the other hand, it is said by Tandler and Kantor ('07) to be the first to appear in the embryological development of the reptilian cortex. Thus practically all the available evidence, whether it be conclusive or not, seems to point in the direction outlined by Kappers.

It would be an interesting observation if it was really the more recent and more highly specialized portions of the cortex which were the less richly vascular, and we have possibly a somewhat similar case among the lower centers studied. Reference to table 3 will show that the chief vestibular nucleus is more highly vascular than the cerebellar cortex, the dentate nucleus, or Deiters' nucleus. Now the cerebellar gray matter is a highly specialized derivative of the primitive acoustico-lateral area, from which, of course, both the other nuclei are also developed. Moreover, the chief vestibular nucleus is composed of small, granule-like cells, most of the axones of which are said to take up a longitudinal direction in the substantia reticularis, so that it seems not unreasonable to suppose that it may be less highly differentiated than either the nucleus of Deiters or the cerebellum. This, however, is pure speculation without definite authority or conclusive basis.

Kappers (*loc. cit.*) offers a suggestion, in another connection altogether, which seems to provide a very plausible explanation, at least in part, for the special vascular richness of the lamina granularis, and perhaps also of the sensory nuclei. In discussing the importance of the granule cells in relation to his principle of neurobiotaxis, he points out that, "while in projection cells the nervous current is directly realized and led away, on the contrary, in the granular cells with short axis cylinders forming an intricate network the stimulation is kept within a certain region." He goes on to point out that the long ascending and descending tracts very often end in relation with such cells, citing among other examples the case of the sensory root fibers ending in the medulla oblongata "and (less general) in the cord." Such a region of concentrated or localized activity might reasonably be expected to have a relatively rich blood supply, and no doubt this is one at any rate of the factors giving rise to the observed differences.

To facilitate comparison of the vascularity of the cerebral cortex with that of the lower centers previously studied, table 3 has been prepared. In this table all the values have been reduced to the basis of a cube of tissue of 100 μ edge, so that the figures given represent the total length of the capillaries in a block of tissue of volume 1,000,000 c. μ , or 0.001 c. mm. This not only makes easy the direct comparison of the figures in the table, but also provides a unit with which comparisons may readily be made in future studies. It may be remarked that the ratios in the table were calculated from the original readings, not from the reduced figures which are tabulated beside them. These ratios show that, on the whole, the vascular supply of the cortex is not much greater than that of the ventral cornu of the gray matter in the spinal cord, but exceeds that of the ventral funiculus of the white matter from three and a half to over seven times.

It will be observed that the vascularity of the insular cortex corresponds roughly with the values obtained for the motor centers, while the various laminae in the other areas cover about the same range as the sensory and correlation centers in the lower

part of the brain, though the richest part of the cortex studied is slightly poorer than the richest center lower down—the dorsal cochlear nucleus. May it be that, great and intense as the activity of the cortex probably is, that activity is nevertheless more intermittent in any one area than is the activity of the lower sensory nuclei? The latter, as was pointed out in the previous paper, are in more or less constant receipt of stimuli, but only a small proportion of these give rise to any reflex, and only a still smaller proportion, when any, reach the cerebral cortex. Many impulses are, no doubt, generated within the cortex itself, and such generation may possibly involve a greater expenditure of energy than the mere passage of an impulse caused by a stimulus somewhere else, so that the cortex may reasonably be expected to be relatively rich in most areas; but the activity in any one portion of the cortex is probably less constant than that in a sensory nucleus, so that greater vascularity is not required.

It might perhaps be mentioned here that what appears to be a clear example of a direct relation between vascularity and functional activity has been described recently in Cajal's laboratory by De Castro ('20), who found such a relation distinctly shown in comparing the vessels belonging to the olfactory glomeruli in man with those in macrosomatic animals.

It may be remarked that, while the 'motor cortex' (*regio praecentralis*) ranks low among the five areas studied, it is very little poorer than the occipital and temporal areas, and is considerably richer than the *regio insularis*.

Finally, we note that the values obtained for the vascularity of the lamina zonalis in the four richer areas are very similar to the figure representing the condition in the molecular layer of the cerebellar cortex.

As in the previous study, the results for the two sexes have been separated and averaged, and the comparison of these is made in the charts in figure 4. The difference between the sexes is much more definite than it was found to be in the lower centers, the vascularity in the males being greater in every lamina of the parietal, temporal, occipital, and praecentral

areas, though the females surpass the males in three of the laminae of the insular cortex. In the subcortical regions the tendency was for the females to be richer than the males. It is perhaps hardly justifiable to base any conclusion upon the averages of so few individuals, especially when the difference is

VASCULARITY

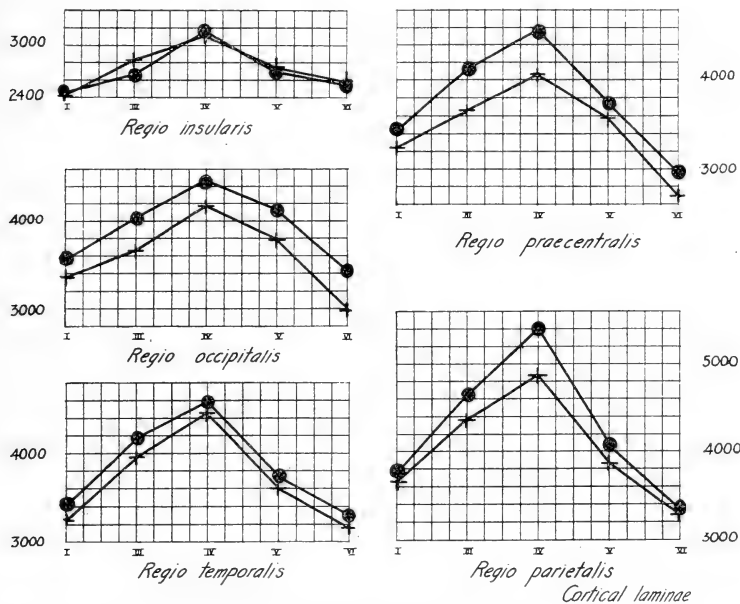


Fig. 4 Graphs showing the relative vascularity of the cerebral cortex of the two sexes in each lamina of the five areas studied. Male ●. Female +.

not very great, being less than the amount of individual variation, and when the poorer group includes the specimen (R. 56) for which the results are suspected of being rather low. Nevertheless, it seems distinctly suggestive that the sexual difference should be so uniform and so much more definite in the cortex cerebri than in the lower centers studied.

A comparison of the groups according to locality has also been made, the average of the results for the four Toronto animals being compared with that for the four specimens from the standard colony of The Wistar Institute. The differences

Vascularity

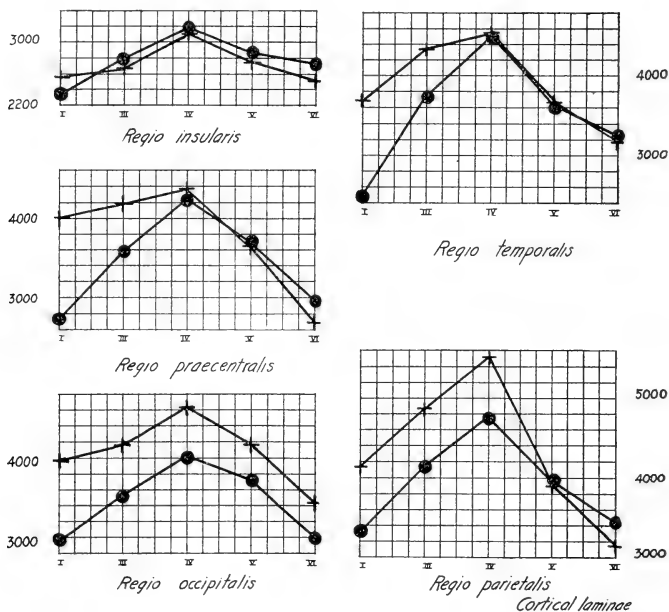


Fig. 5 Graphs showing in the same way as in figure 4 the relative vascularity of the cerebral cortex in rats from two different localities. Toronto ●. The Wistar Institute +.

between these groups are illustrated graphically in figure 5. Here again, the difference is a little more decided than in the lower parts, though less definite than that between the sexes. In this case, however, the suspected specimen (R. 56) belongs to the group which is richer in most layers (the group from The

Wistar Institute). It may be noticed that, in all cases where the Wistar average is less than the Toronto one, it is the deepest layers which are involved, the lamina multiformis being poorer in the Wistar group in four of the five areas, the lamina ganglionaris in three, the laminae granularis interna and pyramidalis in only one, and the lamina zonalis in none. It should be pointed out in this connection that each local group contains two specimens of each sex.

Evidently the difference between the regio insularis and the other four regions is either of some other nature or of a much greater degree than their differences from each other. It is this area which is so much more poorly vascularized than the others; it is this area in which the female average exceeds that of the males in three laminae; and it is this area in which the average for the Wistar animals is poorer in four laminae than that for the Toronto animals.

It should be remarked here that it is possible that some of the differences in the measurements of vascularity are possibly attributable to differences in brain weight. The situation in this respect is summed up by Donaldson³ as follows:

If the vascular supply grows in proportion to the brain—within the limits of variation of brain size in full grown rats—then a sample of a fixed volume of tissue from one brain will be comparable with that from another.

If the increase in the volume of the entire brain is more rapid than that of the vascular supply, then the supply in a fixed volume of tissue will appear less in the larger brain—and vice-versa.

Which of these conditions obtains, the writer is not at present in a position to state definitely, but he is inclined to believe that the second supposition is the one which represents the facts. This would not account, however, for the differences in the relations seen in the different parts of the central nervous system. Unfortunately, the weights of the brains employed in the present study were not recorded, but the body weight and body length of the four rats from The Wistar Institute are known. From these data the probable brain weights may be determined from

³ Personal communication.

the normal tables for the albino rat (Donaldson, '15, table 68), which give the following results:

RAT	SEX	BODY LENGTH	BODY WEIGHT	BRAIN WEIGHT CALCULATED FROM BODY LENGTH	BRAIN WEIGHT CALCULATED FROM BODY WEIGHT
		<i>mm.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
31	♂	200	228.4	1.858	1.900
55	♀	185	159.2	1.782	1.773
56	♀	198	197.4	1.841	1.835
58	♂	210	234.8	1.903	1.907

It is thus evident that the female brains may be regarded as slightly smaller than those of the males. Since the vascularity of the cerebral cortex is poorer in the female brain, rather than richer, the sexual difference cannot be explained upon the basis of size in the manner suggested above.

Since measurements were not recorded for the Toronto animals, a similar scrutiny cannot be applied to the comparison of local groups. It seems not improbable that the difference in this case may be capable of explanation on the above basis, though this would not account for the greater difference found in the cortex as compared with the subcortical regions.

While one must hesitate to generalize upon only two comparisons with the limitations already pointed out, these data seem to suggest a greater susceptibility in the cortical vascularization than in that of the more ancient portions of the central nervous system to differences either within or without the body—sexual, hereditary, or environmental.

The writer is much indebted to Dr. H. H. Donaldson, of The Wistar Institute, who kindly read the manuscript and made a number of valuable suggestions regarding the presentation of the material.

SUMMARY

1. The vascularity of each of the five distinguishable layers in five different areas of the cerebral cortex has been measured in eight albino rat brains out of the ten which were used in the prosecution of a study, already reported (Craigie, '20), upon

the relative vascularity of various parts of the cerebellum, medulla oblongata, and spinal cord.

2. The relative vascularity of the five laminae in a single region is found to be similar in all the cortical areas examined, the lamina granularis interna (IV) being the richest in every case.

3. The supragranular layers show a tendency to be a little richer than the infragranular ones, the poorest layer being the lamina multiformis (VI) in every area except the insular, where the lamina zonalis (I) is very slightly poorer.

4. It seems probable that the granular and supragranular layers are receptive and associative in function, while the infragranular layers give rise to corticifugal fibers. This suggests a comparison with the lower centers, where the sensory and correlation nuclei were found to be more richly vascular than the motor nuclei. Moreover, it has been suggested that the lamina granularis interna is composed of relatively less highly differentiated cells than the other layers, which gives rise to speculation as to why it should be more vascular than the remaining laminae.

5. The average vascularity of the five layers is the same in the occipital and temporal areas, and is only slightly less in the praecentral region. The parietal area is distinctly richer than the others, while the insular cortex is much the poorest.

6. The vascularity of the five laminae in the insular cortex covers about the same range as that of the various motor centers studied in the brain stem and spinal cord (table 3), while the vascularity of the other areas corresponds approximately to that of the sensory and correlation nuclei.

7. The vascularity of the cerebellar cortex is of about the same order of magnitude as that of the cerebral cortex taken as a whole.

8. Sexual and racial differences appear to be more marked in the cortex cerebri than in the parts of the central nervous system previously studied, suggesting that the vascularization of the more recently evolved centers is more susceptible than that of more ancient regions to sexual, hereditary, or environmental influences.

9. The fact that the vascularity of the regio insularis not only is much poorer than that of the other four areas, but also differs from them in its sexual and racial characteristics, seems to indicate that this area differs from the rest more than they do from each other.

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Resumen por el autor, C. Judson Herrick.

Las conexiones del nervio vómero-nasal, bulbo olfatorio accesorio
amígdala en los anfibios.

En los urodelos toda la pared lateral del hemisferio cerebral retiene el carácter del núcleo olfatorio lateral, dentro de cuya parte ventral existe un primordium estrio-amigdalóide no diferenciado. En los anuros esta pared se ha diferenciado en: 1) Un núcleo lateral combinado, formado por el núcleo olfatorio y el lóbulo piriforme; 2) El cuerpo estriado; 3) La amígdala. Los anuros poseen un órgano vómero-nasal bien definido, un nervio vómero-nasal y una formación vomero-nasal en el bulbo olfatorio. La diferenciación de este sistema comienza en los urodelos, pero no ha llegado a consumarse. La amígdala de los anuros se ha desprendido del complejo estrio-amigdalóide de los urodelos bajo la influencia de la diferenciación periférica del órgano vómero-nasal y las vías nerviosas específicas relacionadas con él. En los peces no existe una verdadera amígdala, aun cuando algunos de los elementos representados en este complejo de los cerebros más superiores puede estar presente en varias combinaciones. En los vertebrados más superiores diferentes sistemas funcionales se han agregado a la amígdala primitiva, produciendo complejos amigdalóides de formas diversas, en la mayor parte de los cuales existe un componente olfatorio, aunque puede haberse suprimido como ocurre en el delfín, sin destruir la unidad del complejo. La relación del órgano vómero-nasal con este complejo en los amniotes niotos no ha sido investigada.

Translation by José F. Nonidez
Cornell Medical College, New York

THE CONNECTIONS OF THE VOMERONASAL NERVE, ACCESSORY OLFACTORY BULB AND AMYGDALA IN AMPHIBIA

C. JUDSON HERRICK

Anatomical Laboratory, The University of Chicago

THIRTY-SEVEN FIGURES

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I. INTRODUCTION

It has long been known that in various mammals, reptiles, and amphibians the olfactory bulb is divided into two parts or lobules, of which the smaller, usually called the accessory bulb, occupies various positions, generally near the caudal border of the main bulb. It was early recognized by a few students of this region that the accessory bulb is related by a special strand of nerve fibers with the vomeronasal organ (Jacobson's organ) of the nose; and this morphological relationship has more recently been emphasized by McCotter ('12). In 1917 McCotter extended his previous observations on the mammals to include the turtle and the frog.

From all of these studies it is clear that the neuro-epithelium of the vomeronasal organ is similar in character to that of the remainder of the olfactory mucosa; that from it there arises in amniote vertebrates a special nerve, the vomeronasal nerve, which is morphologically and probably physiologically a specialized part of the olfactory nerve, and that the vomeronasal nerve terminates in a differentiated portion of the olfactory bulb, the

accessory bulb. The nervus terminalis is distinct from both the olfactory nerve and the vomeronasal nerve, as has been clearly shown by McCotter ('13) and others.

The portion of the olfactory bulb which receives the fibers from the general olfactory epithelium is termed by McCotter ('13) the olfactory formation (regio bulbaris principalis of Gaupp, '99, in the frog); the portion which receives the vomeronasal nerve (i. e., the accessory bulb) is the vomeronasal formation (Zuckermandl, '10, p. 11). The latter is usually small, but in the turtle (*Chrysemys*) it is nearly as large as the olfactory formation, occupying the entire dorsal surface and upper half of the medial surface of the olfactory bulb (McCotter, '17). The vomeronasal nerve and formation appear to vary in size in proportion to the extent of the sensory epithelium of the vomeronasal organ (Zuckermandl, 10 a, p. 839).

In a review of the relations of the vomeronasal organ to the respiratory mechanisms of Amphibia, Bruner ('14) has summarized some of the literature and discussed the functional relations. He supports the belief of Seydel that in the higher amphibians the nasal organ consists of two functionally and morphologically distinct parts; a true olfactory cavity which is used primarily for testing the inspiratory current, and an organ of Jacobson which receives its stimuli through the medium of the expiratory current. His detailed examination of the respiratory mechanisms and their functional workings in various amphibians shows that this relationship prevails whether the olfactory medium be air or water.

Bruner distinguishes two types of respiration in Amphibia:

a) In the first the respiratory medium passes freely inward through the nasal cavity to the mouth, but its return to the nasal cavity from the mouth is prevented by a mechanical valve at the choana. b) In the second type the respiratory mechanism is wholly under muscular control and the olfactory medium passes freely in and out through the nasal cavity.

Corresponding to these two types of the respiratory mechanism, we can distinguish a) Monosmatic forms (single smellers), including *Necturus* and the larvae of *Amblystoma* and *Rana*, in which the olfactory organ is used to test only the external medium. For this condition I propose the name monosmesis. b) Diosmatic forms (double smellers),

including *Siren*, *Cryptobranchus*, *Amphiuma*, larvae of lungless salamanders, and the adult stage of higher amphibians. In these forms, ingoing and outgoing currents bear odorous matter to the olfactory organ, which is accordingly used to test both the external medium and the contents of the oral cavity. This condition, which is common to all higher vertebrates, may be called diosmesis.

In (adult)¹ single smellers (*Necturus*)¹ Jacobson's organ is wanting and the olfactory organ has a very simple condition. In double smellers the olfactory organ is complex and Jacobson's organ is present.

For the development of a complex olfactory organ, with an organ of Jacobson, the nature of the olfactory medium is of less importance than the question whether the animal is a single or a double smeller.

The more recent observations of Broman ('20) on reptiles and mammals are commented upon on page 265.

In the frog the vomeronasal formation (*bulbus olfactorius accessorius* of Gaupp, '99, p. 99) forms a distinct accessory bulb on the lateral surface of the cerebral hemisphere farther caudad than the olfactory formation. Its relations to the vomeronasal nerve and organ have been fully described by McCotter ('17).

In 1910 in connection with a brief review of the forebrain of the frog I described a tract of unmyelinated fibers, the ventrolateral olfactory tract ('10, pp. 422, 444 and figs. 40, 41), which passes from the accessory olfactory bulb to the so-called corpus striatum, or lateral basal nucleus of the hemisphere. In view of the peripheral relationship of the accessory olfactory bulb to the vomeronasal organ, it is clear that the ventrolateral olfactory tract can be nothing other than the central conduction pathway of the vomeronasal system, and the terminal nucleus of this tract, a part of the so-called corpus striatum of the frog, is similarly related to the vomeronasal apparatus.

The fiber connections of the region hitherto called corpus striatum in the frog show that it must be separated into two clearly distinct parts: 1) a true corpus striatum (*paleostriatum*) which is very simply developed in the frog, and, 2) farther caudad a definite nucleus which is comparable with a part of the mammalian nucleus amygdalae. The latter nucleus receives

¹ The words in parenthesis are added with a pen in the copy of the author's reprint sent to me.

the ventrolateral olfactory tract from the vomeronasal formation and will be here termed the amygdala. Our attention in this paper will be chiefly directed to the structure and connections of the amphibian amygdala.

The terminology of the amphibian forebrain is in great confusion. The usage here employed conforms in general, except as noted, with that of my contribution published in 1910.

The cerebral hemisphere of the frog is divisible into five regions, the olfactory bulb and four quadrants termed dorsomedial, ventromedial, ventrolateral, and dorsolateral. The dorsomedial quadrant is the primordial hippocampus; the ventromedial quadrant is the septum, using this term in its broadest sense; the ventrolateral quadrant includes the very undifferentiated corpus striatum and the amygdala, which is a sharply defined nucleus; the dorsolateral quadrant comprises the lateral olfactory nucleus and the pyriform lobe, which are relatively undifferentiated and not clearly separable from each other.

All four quadrants converge into an undifferentiated anterior olfactory nucleus at the base of the olfactory bulb, and the two dorsal quadrants converge into an undifferentiated area at the posterior pole of the hemisphere. Comparison with higher brains shows that the dorsal quadrants are primordial pallium (the dorsolateral incompletely so), but the ventral quadrants are essentially basal nuclei and do not in any vertebrate give rise to true cerebral cortex. The general relations of the amphibian cerebral hemispheres to those of other ichthyopsid forms are reviewed in a recent paper (Herrick, '21).

This account is based upon a collection of several hundred brains of urodele and anuran Amphibia prepared by various methods by Dr. Paul S. McKibben, upon about 150 brains of larval *Amblystoma* prepared by the Golgi method by Dr. Charles Brookover, and upon about 150 brains of adult and larval *Amblystoma* variously prepared in this laboratory by my assistant, Miss Jeannette B. Obenchain. To these careful workers I acknowledge my deep indebtedness.

II. ANURA

The olfactory bulb

In the frog the internal structure of the vomeronasal formation is, so far as observed, essentially similar to that of the remainder of the olfactory bulb. The fibers of the vomeronasal nerve enter glomeruli, where they engage dendrites of the mitral cells in the usual way. Beneath the mitral cells is a layer of granule cells bordering the ventricle (figs. 1, 19).

The accessory bulb not only forms the familiar eminence on the lateral surface of the cerebral hemisphere, but also projects into the lateral ventricle as a well-defined ventricular swelling (figs. 1, 35). This is shown, somewhat imperfectly, in horizontal section in figures by P. Ramón y Cajal ('05, pl. 15, fig. 3, and pl. 17, fig. 5). Through the deeper part of this thickening there pass fibers of the dorsolateral olfactory tract (a few of which are myelinated) on their way from the ventral part of the olfactory formation to their definitive position in the dorsolateral quadrant of the hemisphere. Toward the caudal end of the accessory bulb these fibers form a distinct fascicle at its dorsomedial angle (fig. 1).

In the olfactory formation of the bulb the layer of granule cells is separated from the layer of mitral cells by a distinct molecular layer; this latter layer, however, is absent in the vomeronasal formation (fig. 19), and here there is no clear boundary between mitral cells and granule cells.

The ventrolateral olfactory tract

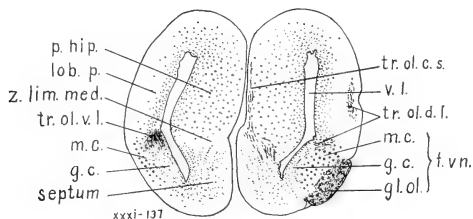
The course of the ventrolateral olfactory tract, its termination in the amygdala, and the course of the dorsal olfactory projection tract from the latter organ to its nucleus in the chiasma ridge as seen in cross-sections of the brain of the frog, *Rana pipiens*, are illustrated in figures 1 to 12. These figures are drawn from a series of Weigert sections and for purposes of orientation all myelinated fiber tracts are entered on the right side of the drawings. The greater part of the courses of the ventrolateral olfactory tract and the olfactory projection tract can be followed in

ABBREVIATIONS FOR ALL FIGURES

<i>amg.</i> , nucleus amygdalae	<i>nuc.ac.</i> , nucleus accumbens septi
<i>b.ol.ac.</i> , bulbus olfactorius accessorius	<i>nuc.ol.ant.</i> , nucleus olfactorius anterior
<i>c.f.</i> , columna fornicis	<i>nuc.o.p.tr.</i> , nucleus of dorsal olfactory projection tract
<i>c.gen.lat.</i> , corpus geniculatum laterale	<i>nuc.po.</i> , nucleus preopticus
<i>ch.</i> , chiasma opticum	<i>nuc.v.l.</i> , nucleus ventrolateralis hemisphaerii
<i>ch.r.</i> , chiasma ridge	<i>p.d.hyth.</i> , pars dorsalis hypothalami
<i>com.amg.</i> , commissura amygdalarum	<i>p.f.</i> , prominencia fascicularis
<i>com.ant.</i> , commissura anterior	<i>p.hip.</i> , primordium hippocampi
<i>com.hab.</i> , commissura habenularum	<i>pol.post.</i> , posterior pole of cerebral hemisphere
<i>com.hip.</i> , commissura hippocampi	<i>p.v.hyth.</i> , pars ventralis hypothalami
<i>com.po.</i> , commissura postoptica	<i>p.v.l.h.</i> , pars ventrolateralis hemisphaerii
<i>c.s.</i> , corpus striatum	<i>p.v.th.</i> , pars ventralis thalami
<i>d.amg.hab.</i> , decussation of tractus amygdalo-habenularis	<i>r.po.</i> , recessus preopticus
<i>d.B.</i> , diagonal band of Broca	<i>s.a.</i> , stratum album
<i>d.f.lat.t.</i> , decussation of lateral forebrain bundles	<i>sac.v.</i> , saccus vasculosus
<i>d.f.med.t.</i> , decussation of medial forebrain bundles	<i>sep.ep.</i> , septum ependymale
<i>d.ol.p.tr.</i> , dorsal olfactory projection tract	<i>s.g.</i> , stratum griseum
<i>d.tr.th.h.c.</i> , decussation of tractus thalamo-hypothalamicus cruciatus	<i>str.med.</i> , stria medullaris
<i>em.th.</i> , eminentia thalami	<i>str.t.</i> , stria terminalis
<i>ep.</i> , epiphysis	<i>tect.</i> , tectum mesencephali
<i>epen.</i> , ependyma	<i>thal.</i> , thalamus
<i>F.</i> , foramen interventriculare	<i>tr.amg.hab.</i> , tractus amygdalo-habenularis
<i>fm.</i> , fimbria	<i>tr.amg.p.</i> , tractus amygdalo-piriformis
<i>f.lat.t.</i> , fasciculus lateralis telencephali (lateral forebrain bundle)	<i>tr.c.hab.l.</i> , tractus cortico-habenularis lateralis
<i>f.med.t.</i> , fasciculus medialis telencephali (medial forebrain bundle)	<i>tr.ol.c.s.</i> , tractus olfacto-corticalis septi
<i>f.ol.</i> , formatio olfactoria	<i>tr.ol.d.l.</i> , tractus olfactorius dorsolateralis
<i>f.retr.</i> , fasciculus retroflexus Meynerti	<i>tr.ol.hab.ant.</i> , tractus olfacto-habenularis anterior
<i>f.vn.</i> , formatio vomeronasalis	<i>tr.ol.v.l.</i> , tractus olfactorius ventrolateralis
<i>g.c.</i> , granule cells	<i>tr.op.</i> , tractus opticus
<i>gl.ol.</i> , glomeruli olfactorii	<i>tr.s.hab.</i> , tractus septo-habenularis
<i>hab.</i> , habenula	<i>tr.th.f.</i> , tractus thalamo-frontalis
<i>hyp.g.</i> , hypophysis, pars glandularis	<i>tr.th.h.c.</i> , tractus thalamo-hypothalamicus cruciatus
<i>hyp.n.</i> , hypophysis, pars nervosa	<i>tr.th.p.i.</i> , tractus thalamo-peduncularis intermedius
<i>inf.</i> , infundibulum	<i>v.l.</i> , ventriculus lateralis
<i>lob.p.</i> , lobus piriformis	<i>v.ol.p.tr.</i> , ventral olfactory projection tract
<i>l.t.</i> , lamina terminalis	<i>v.3.</i> , third ventricle
<i>m.b.</i> , midbrain	<i>z.lim.lat.</i> , zona limitans lateralis
<i>m.c.</i> , mitral cells	<i>z.lim.med.</i> , zona limitans medialis
<i>mol.</i> , stratum moleculare	
<i>n.ol.</i> , nervus olfactorius	
<i>n.ol.lat.</i> , lateral division of olfactory nerve	
<i>n.op.</i> , nervus opticus	
<i>n.term.</i> , nervus terminalis	

these sections, though their fibers are unmyelinated. These are sketched in on the left side of the drawings, their terminal relations (which are not demonstrated by the Weigert sections) being added from data derived from Golgi sections in various planes.

These connections are also shown as seen in horizontal sections by the Golgi method of the cricket frog, *Acris gryllus*, in figures



Figs. 1 to 12. A series of transverse sections through the brain of *Rana pipiens*. $\times 12$. The outlines and arrangement of cells and myelinated fibers are drawn to scale from a series of Weigert sections. The sections are somewhat compressed dorsoventrally and are slightly oblique, the right side being farther rostral than the left. The arrangement of cells is necessarily somewhat conventionalized on account of the small scale of the drawings.

These sections were prepared by the Weigert method, fixing in formalin and potassium bichromate, mordanting the sections in copper acetate, and destaining in potassium permanganate and the oxalic potassium sulphite mixture. The destaining was arrested at a point which differentiated the myelinated fibers, but left considerable brownish-yellow color in the background. All cell bodies are clearly visible and their arrangement was further controlled by comparison with sections stained with toluidin blue. Some of the unmyelinated tracts are also differentiated by the 'brown reaction.'

The myelinated fibers are entered only on the right side of the drawings. On the left side are entered the ventrolateral olfactory tract and the dorsal olfactory projection tract, both of which are unmyelinated. The courses of these tracts can be followed in the Weigert sections throughout their length except near their ends, but the details of these unmyelinated fibers are drawn in by comparison with various other series of transverse and longitudinal sections prepared by the methods of Golgi and Cajal.

Fig. 1. On the left the section passes through the extreme caudal end of the vomeronasal formation, showing its layers of granule cells and mitral cells. There are no glomeruli at this level; on the right these are present. The unmyelinated fibers of the ventrolateral olfactory tract have assembled from the vomeronasal formation to form a compact bundle in the deeper part of the zona limitans lateralis.

19 to 21. We have sections of the brains of several other specimens of different species of frogs prepared by the methods of Golgi and Cajal and cut in various oblique longitudinal planes in which each of these tracts is clearly demonstrated for practically its entire length in a single section.

Since the cell bodies from which these fibers arise are not impregnated in these preparations, the direction of conduction is not demonstrated. The indications are, however, clear that the ventrolateral olfactory tract is mainly (probably exclusively) a descending system from the vomeronasal formation to the amygdala and free terminal arborizations of these fibers are seen

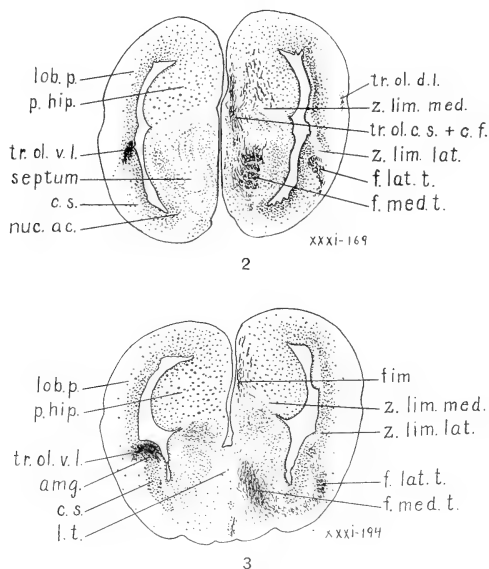


Fig. 2 Through the cerebral hemisphere between the vomeronasal formation and the lamina terminalis. The ventrolateral olfactory tract lies close to the ventricle in the zona limitans lateralis.

Fig. 3 Through the rostral border of the lamina terminalis. On the left the ventrolateral olfactory tract is seen entering the rostral end of the amygdala.

in the amygdala. There is no evidence of physiological connection by collateral branches or terminals with any other neurons between its origin in the vomeronasal formation and its termination in the amygdala. That is, the true corpus striatum of the Anura does not receive any appreciable number of olfactory fibers, a relation in marked contrast with that of Urodela (p. 242).

The ventrolateral olfactory tract rises up slightly dorsalward from its origin and then passes directly backward in the deeper layers of the zona limitans lateralis between the dorsal and the

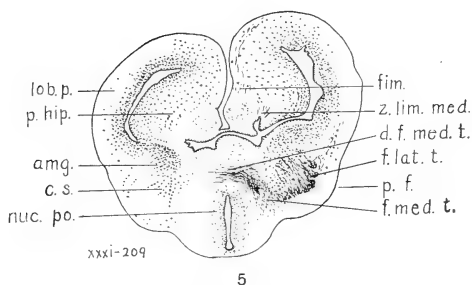
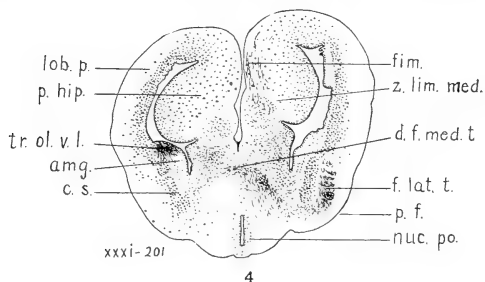
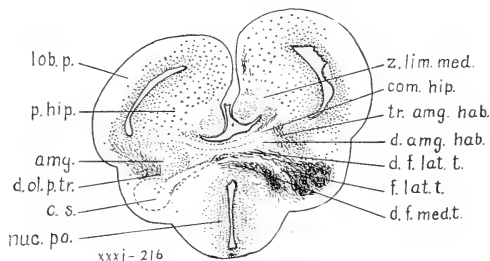


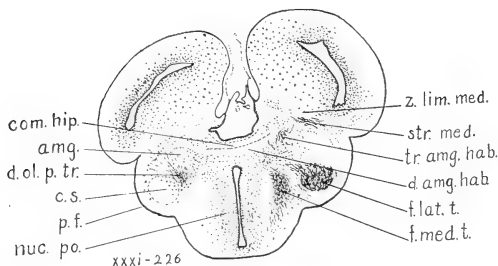
Fig. 4 Through the lamina terminalis immediately rostral to the interventricular foramen, showing terminals of the ventrolateral olfactory tract in the dorsorostral part of the amygdala.

Fig. 5 Through the interventricular foramen on the right side and immediately behind it on the left, the section being slightly oblique. At this level the amygdala attains its maximum size and its neurons are very-numerous; those of the corpus striatum are few in number.

ventral-lateral quadrants of the cerebral hemisphere. Upon reaching the amygdala its fibers turn ventralward to arborize freely among the neurons of the dorsolateral part of this nucleus.



6



7

Fig. 6 Through the middle of the anterior commissure complex. At this level there are represented in this commissural complex the decussation of the medial forebrain bundles, the decussation of the lateral forebrain bundles, the commissura hippocampi, and between the last two a mixed system of unmyelinated fibers containing the commissure of the two amygdalae and habenular fibers (cf. fig. 17 and p. 227). Unmyelinated fibers of the dorsal olfactory projection tract are present in the lateral part of the amygdala. A few myelinated fibers of the habenular tract of the amygdala (tractus amygdalo-habenularis) are seen on the right.

Fig. 7 Through the caudal end of the anterior commissure ridge, containing unmyelinated fibers of the hippocampal commissure. The fibers of the dorsal olfactory projection tract are leaving the ventral border of the amygdala. The amygdalo-habenular tract is directed upward toward the stria medullaris. The section passes through the extreme caudal end of the corpus striatum, represented by a few neurons embedded in the lateral forebrain bundle.

The structure and connections of the amygdala

The amygdala is anatomically one of the most clearly defined regions of the anuran brain. Externally it participates somewhat in the formation of the ventrolateral ridge termed by Gaupp *prominentia fascicularis*, though this eminence is formed chiefly by the lateral forebrain bundle and the associated corpus striatum. The amygdala is somewhat pear-shaped, the stem of the pear (which is directed forward) receiving the ventrolateral olfactory tract. Internally it is ovoid in cross-section and projects into the lateral ventricle at the level of the interventricular foramen (figs. 5 and 17).

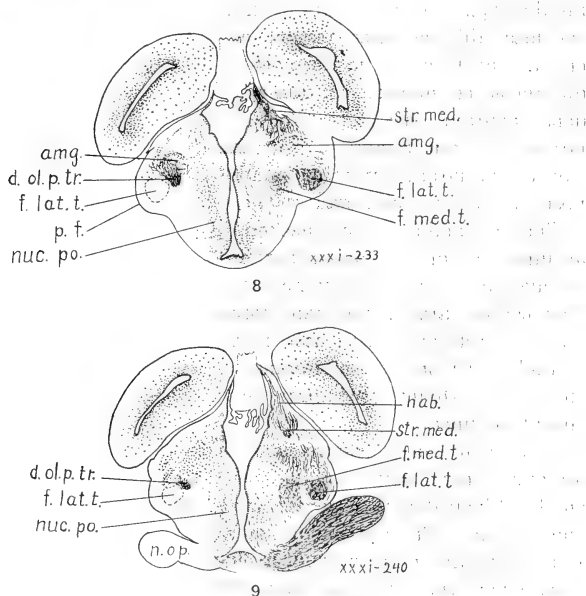


Fig. 8 Through the extreme caudal end of the amygdala.

Fig. 9 Through the rostral end of the thalamus immediately behind the amygdala.

This part of the frog brain has been called corpus striatum by some previous writers (including myself, '10). But not by Gaupp ('99, p. 107), who with his usual acumen recognizes that only the undifferentiated middle part of the ventrolateral area which is closely associated with the lateral forebrain bundle is with certainty to be compared with the 'basal ganglia' of higher vertebrates. Further than this he was not able to carry his analysis of this region.

The true corpus striatum of the frog, as already indicated, occupies the ventrolateral quadrant of the cerebral hemisphere, chiefly rostrally of the interventricular foramen, and is characterized by its functional connection with the lateral forebrain bundle (figs. 2 to 6, 14, 15). Its more rostral portion is one of the least differentiated regions of the hemisphere. Its caudal end at the level of the foramen is expanded to form a highly differentiated nucleus containing but few neurons and a very dense neuropil (figs. 5, 6, 17, 20, 23) derived chiefly from the lateral forebrain bundle. From this neuropil arises the decussation of the lateral forebrain bundle (figs. 6, 17), which is composed largely of collaterals of the fibers of this bundle.

The cell bodies of the neurons of the amygdala tend to be accumulated on the medial border, though some are distributed throughout. They are sparse or absent on the lateral side. This cellular area is confluent dorsally with that of the pyriform lobe, ventrolaterally with that of the corpus striatum, and ventromedially with that of the lamina terminalis and preoptic nucleus (figs. 4, 5), though the limits between these regions are rather sharply defined.

As seen in Golgi preparations (fig. 13), some neurons of the amygdala have large rounded cell bodies and some are very small. Their dendrites spread widely and irregularly throughout the nucleus. The neurons of the adjacent corpus striatum tend to be larger and more irregular in shape. In most of our preparations where the cell bodies and dendrites are impregnated their axons are not, and conversely, so that it is difficult to be sure of the connections of individual cells. The impression gained from the preparations is that the larger elements send their

axons downward into the olfactory projection tracts, while the axons of the smaller ones are directed laterally and dorsally into the pyriform lobe (tractus amygdalo-pyriformis).

In Golgi preparations in which the cell bodies and dendrites of none of the intrinsic neurons of the amygdala are impregnated the entire structure is filled with a very dense neuropil of fine fibers. These are axons, apparently chiefly of extrinsic origin, and this neuropil may be divided into two incompletely separable parts, one at the rostral end related chiefly with the ventro-lateral olfactory tract, and below this a larger part related with the hypothalamic, habenular, and commissural connections.

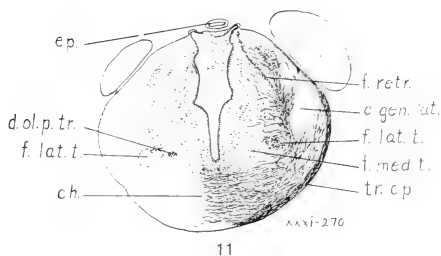
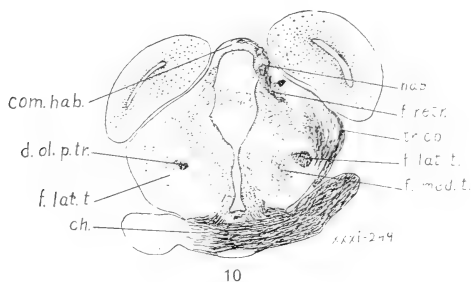


Fig. 10 Through the habenular commissure and the rostral border of the optic chiasma. The olfactory projection tract is passing ventralward, medialward, and spinalward along the medial border of the lateral forebrain bundle.

Fig. 11 Through the caudal part of the chiasma ridge and postoptic commissure complex and the posterior poles of the cerebral hemispheres.

The amygdala of the frog, as we have seen, receives a direct olfactory tract of the second order from the vomeronasal formation, the tractus olfactorius ventrolateralis, and is therefore to be regarded as a derivative of the primitive lateral olfactory nucleus. It has in addition the following fiber connections, all of which are characteristic of regions of both lower and higher brains related with the olfactory complex:

1. A commissural strand in the anterior commissure connected with the amygdala of the opposite side.

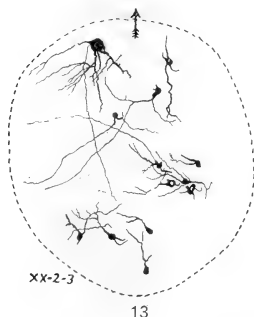
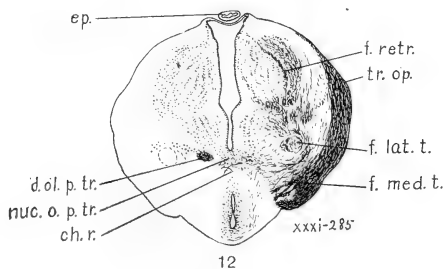


Fig. 12 Through the caudal edge of the chiasma ridge, including the nucleus of the olfactory projection tract.

Fig. 13 Neurons of the amygdala of *Rana catesbiana*. Golgi method. $\times 70$. The section is from a horizontal series, left side, at the level of the inter-ventricular foramen; cf. figure 19. The broken line marks the outline of the amygdala, the arrow points rostrad, and the medial surface is at the right.

2. An amygdalo-habenular connection by way of the stria medullaris, some of these fibers decussating in the anterior commissure.

3. An amygdalo-pyriform connection with the overlying pyriform lobe.

4. The diagonal band of Broca, directed forward and medialward under the lateral forebrain bundle to connect with the septum.

5. The stria terminalis, directed forward and medialward above the lateral forebrain bundle, to connect with the septum and preoptic nucleus, some of its fibers probably decussating in the anterior commissure.

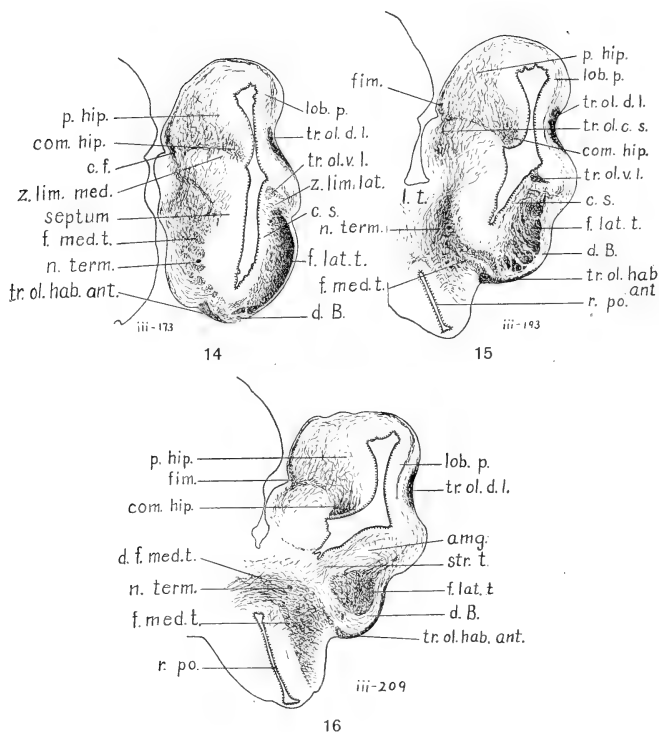
6. The ventral olfactory projection tract, directed backward and medialward under the lateral forebrain bundle.

7. The dorsal olfactory projection tract, directed backward and medialward above the lateral forebrain bundle, to connect with the hypothalamus.

These seven connections will next be described. They are schematically indicated in figures 35 and 36.

The commissura amygdalarum. This is a very small compact strand of unmyelinated fibers passing between the two amygdalae in the anterior commissure (figs. 17, 20). It lies ventrally of the decussations of the amygdalo-habenular tracts and lateral forebrain bundles and dorsally of that of the medial forebrain bundle. In Golgi preparations its fibers are lost in the dense neuropil of the amygdala and their cells of origin are not demonstrated.

Tractus amygdalo-habenularis. This is a system of fibers, partly myelinated (figs. 6, 7) and partly unmyelinated (figs. 17, 18), between the amygdala and the habenula. They accumulate on the dorsomedial border of the amygdala and pass directly dorsalward to enter the stria medullaris. Some of them appear to decussate in the anterior commissure dorsally of the commissure of the amygdalae and decussation of the lateral forebrain bundles and ventrally of the hippocampal commissure (fig. 17, *d. amg. hab.*). This component of the anterior commissure is of open texture and its fibers are diffusely arranged, in marked contrast with the other three components of this commissure complex last mentioned.



Figs. 14 to 18 Five transverse sections through the brain of *Rana pipiens* prepared by the silver method of Cajal. $\times 20$. The specimens were much distorted by shrinkage during preparation, as will be seen by comparison with figures 1 to 12.

Fig. 14 Section taken a short distance rostral to the lamina terminalis between the levels of figures 2 and 3.

Fig. 15 Section through the rostral end of the lamina terminalis between the levels of figures 3 and 4.

Fig. 16 Section immediately rostral to the interventricular foramen at about the level of figure 4. The amygdala forms an eminence projecting into the lateral ventricle. It is connected with the ventromedial olfactory centers by the stria terminalis above the lateral forebrain bundle and by the diagonal band of Broca below this bundle.

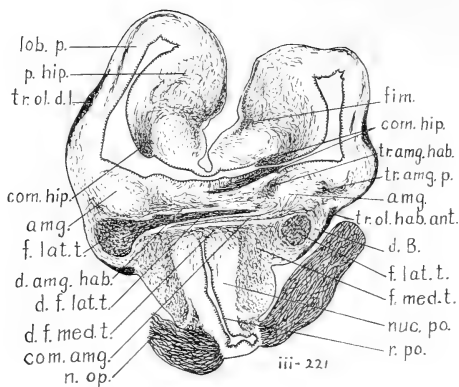
Mingled with the fibers of the amygdalo-habenular tract are others passing between the septum and the habenula (fig. 18, *tr. s. hab.*), the tractus septo-habenularis of my earlier account ('10, pp. 426, 428). Some of these fibers apparently decussate in the anterior commissure in company with those of the tractus amygdalo-habenularis.

The tractus amygdalo-habenularis of this description was designated in the former paper ('10, pp. 429, 444) tractus habenulo-striaticus. Our preparations do not indicate the direction of conduction of its fibers. This tract contains the only myelinated fibers related with the amygdala of the frog. The tractus strio-thalamicus which I mentioned ('10, p. 444) as related with this region is connected with the true corpus striatum (whose limits at that time were not clearly defined) and has nothing to do with the amygdala.

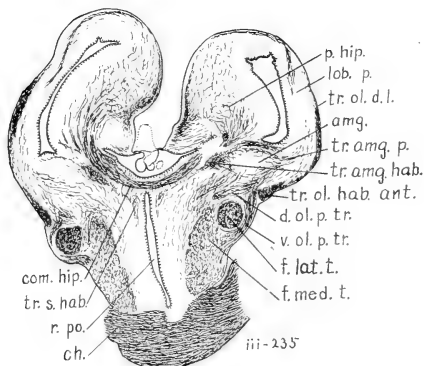
NOTE ON THE TRACTUS OLFACTO-HABENULARIS ANTERIOR. In all species of Amphibia which I have studied there is a connection (which was overlooked in my paper published in 1910) between the ventral part of the septal region near its rostral end and the habenula, which I shall term the tractus olfacto-habenularis anterior. This clearly defined tract of unmyelinated fibers passes caudad along the extreme ventral surface of the cerebral hemisphere, and at the rostral end of the lamina terminalis occupies the angle between this structure and the ventral border of the prominentia fascicularis (figs. 14 to 18). It then turns abruptly dorsad and caudad to join the tractus cortico-habenularis lateralis in the angle between the dorsal border of the prominentia fascicularis and the posterior pole of the hemisphere. In the latter part of its course it is a thin sheet of fibers forming the most superficial layer of the prominentia fascicularis. The less densely stained fibers of the diagonal band form an intermediate layer between these and the longitudinally directed fibers of the lateral forebrain bundle.

Tractus amygdalo-piriformis. There is a broad connection between the amygdala and the overlying pyriform lobe, probably of mixed character. For the observed relations see the account of the dorsal olfactory projection tract below.

The diagonal band of Broca. From the entire lateral surface of the amygdala very fine unmyelinated fibers assemble in diffuse formation and turn ventralward in a narrow zone external to the lateral forebrain bundle and, farther forward, ventrally of it.



17



18

Fig. 17 Section through the interventricular foramen and anterior commissure; cf. figures 5 and 6. The amygdala is seen to be connected with the pyriform lobe by amygdalo-pyriform fibers and with the opposite side by two systems of fibers, one above and one below the decussation of the lateral fore-brain bundles. The ventral system, commissura amygdalarum (*com. amg.*), is a small compact fascicle of unmyelinated fibers which apparently form a true commissure. The dorsal system is a more diffuse collection of fibers of various sorts, containing decussating fibers of the tractus amygdalo-habenularis (*d. amg. hab.*), mingled with those of the tractus septo-habenularis and perhaps other kinds of fibers. Amygdalo-habenular fibers are accumulating on the dorso-medial border of the amygdala; these are both myelinated and unmyelinated.

Fig. 18 Section immediately caudad of the interventricular foramen at about the level of figure 7. Fibers of the olfactory projection system are passing ventralward, medialward, and spinalward from the amygdala, some dorsally of the lateral forebrain bundle (*d. ol. p. tr.*) and some ventrally of this bundle (*v. ol. p. tr.*).

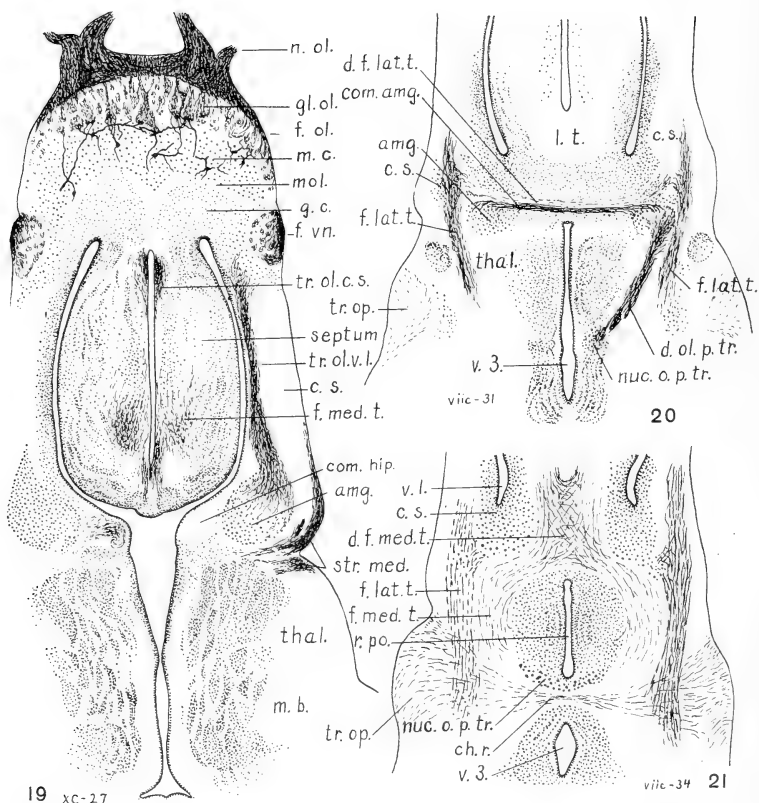
Here they form a compact sheet of fibers directed ventralward, medialward, and forward. Some of them apparently connect with the preoptic nucleus; others extend farther forward to connect with the septal nuclei (figs. 14 to 17).

Stria terminalis. There is another connection of unmyelinated fibers between the amygdala and the medial olfactory areas of the septum and preoptic nucleus. These fibers leave the medial surface of the amygdala near its rostral end and pass medialward and forward dorsally and medially of the lateral forebrain bundle, forming the stria terminalis (fig. 16). The diagonal band and the stria terminalis are best seen in our Cajal sections. The direction of conduction is not revealed in these preparations. The fibers of both tracts probably run in both directions between the amygdala and the medial olfactory areas. Some fibers of the stria terminalis system probably decussate in the anterior commissure, though our preparations do not demonstrate this.

The ventral olfactory projection tract. These fibers (fig. 18) occupy the same position as the diagonal band of Broca farther rostrad, these two systems forming a single sheet of fibers running superficially across the prominentia fascicularis and distinguished only by the fact that the fibers of the diagonal band are directed forward, while those of the projection tract are directed ventralward and probably spinalward. The latter join the medial forebrain bundle, and their destination is unknown. They probably connect with the adjacent preoptic nucleus and perhaps with the hypothalamus. The direction of conduction has not been determined.

The dorsal olfactory projection tract. This small but very well-defined tract takes a course similar to the last, except that it runs dorsally and medially of the lateral forebrain bundle and can definitely be related to a specific nucleus of the hypothalamus.

Almost the entire course of this tract can be seen in a single section in some of our Golgi preparations, as illustrated in horizontal section of *Acris gryllus* in figure 20 and in an oblique plane in *Rana pipiens* in figures 22 and 23. For the relations in transverse sections see figures 6 to 12. Its fibers take a very direct course between the amygdala and the gray matter near



Figs. 19 to 21 Three horizontal sections through the brain of *Acris gryllus*. Golgi method. $\times 35$. Figure 19 is from a specimen in which the ventrolateral olfactory tract is impregnated for its entire length, and figures 20 and 21 are from another specimen cut in the same plane, in which the olfactory projection tract is impregnated, in both cases on the right side only. The cell bodies of but few neurons are impregnated in these preparations. Those which are unimpregnated, however, are clearly visible and their arrangement is indicated somewhat conventionally by stipple.

the dorsal border of the chiasma ridge, which is termed the nucleus of this tract. Our preparations indicate that this tract includes both ascending and descending fibers in both *Anura* and *Urodela*. Free terminal arborizations are abundant at both ends of the tract and in urodeles axons of neurons of its hypothalamic nucleus are directed dorsally into it.

The nucleus of the olfactory projection tract is not clearly defined in cell preparations (figs. 12, 20, 21), but in Golgi preparations of *Rana* it is seen as a horseshoe-shaped mass of dense neuropil occupying the most dorsal part of the chiasma ridge immediately caudad of the decussating chiasma fibers and extending across the median plane (figs. 22, 23). In *Anura* it appears to occupy the extreme dorsal border of the chiasma ridge, but in urodeles it lies considerably farther caudad and ventrad in the hypothalamus. We have no satisfactory impregnation of its neurons in the *Anura*.

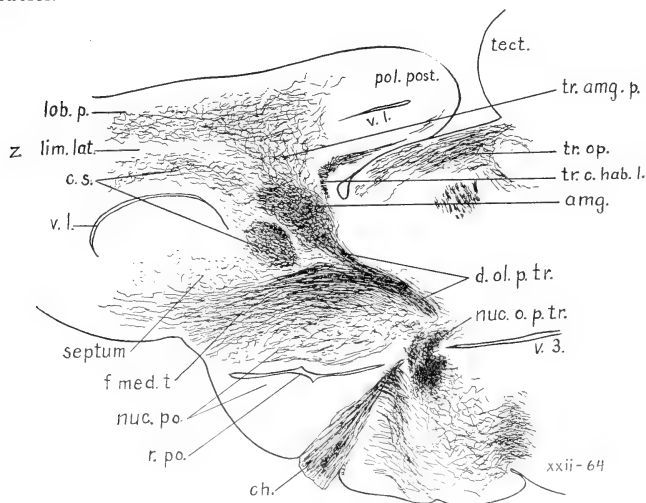
Following the dorsal olfactory projection tract spinalward from the amygdala (figs. 7 to 12), its fibers are seen to assemble from the entire caudal part of this structure on the medial border of the lateral forebrain bundle, to accompany this bundle for

Fig. 19 Section through the olfactory and vomeronasal formations of the olfactory bulb and the amygdala. The ventrolateral olfactory tract, which curves slightly dorsally in its course from the vomeronasal formation to the amygdala, does not lie in the plane of this section. On the right side it is sketched in by projection from the three adjacent sections farther dorsal. In the region of the vomeronasal formation the stratum moleculare is absent and the mitral cells are more or less mingled with the granule cells.

Fig. 20 The section passes considerably farther ventral than that shown in figure 19, below the level of the interventricular foramen. The amygdala lies for the most part dorsally of this level. From its ventral border the olfactory projection tract passes backward and medialward to its nucleus at the level of the chiasma ridge. The brain being small and the sections thick, almost the whole length of this tract lies in the plane of a single section. At its caudal end it dips ventralward for a short distance. The commissure between the amygdalae and the decussation of the lateral forebrain bundles are sketched in from the adjacent section ventrally.

Fig. 21 Three sections ventrally of the plane of figure 20, passing through the dorsal border of the chiasma ridge. The nucleus of the olfactory projection tract lies mostly dorsally of this plane, probably including some neurons in the position indicated (*nuc.o.p.tr.*) at the level of this section. Its boundaries are nowhere clearly defined in this series.

a short distance and then diverge from it medialward and ventralward to connect with its nucleus in the chiasma ridge. Its fibers are probably derived in part from the large neurons of the amygdala (fig. 13) and in part from the nucleus at its lower end. Free terminal arborizations are found in both of its terminal nuclei.



Figs. 22 and 23 Two adjacent longitudinal sections through the brain of *Rana pipiens* by the Golgi method. $\times 20$. The plane of section is inclined about 45° to the horizontal, as indicated by the diagram, figure 23 A, and the caudal ends of the sections figured are, on the right side, slightly nearer the median plane than the rostral ends. The sections are taken from the right side, including a portion of the left side below.

The plane of these sections is so chosen as to show the entire extent of the dorsal olfactory projection tract and its nucleus, a horseshoe-shaped neuropil lying on both sides of the meson in the dorsal part of the chiasma ridge. The arborizations of this tract in the dorsolateral quadrant (pyriform lobe) of the cerebral hemisphere are shown. There are also included a portion of the lateral forebrain bundle, the neuropil of the corpus striatum from which its decussating fibers arise, the forward extension of this bundle into the ventrolateral quadrant (corpus striatum), and a portion of the medial forebrain bundle extending forward into the ventromedial quadrant (septum).

Some of the ascending fibers of this tract end by free arborization in the amygdala, but many of them pass along the caudal border of this nucleus, giving off collaterals to its neuropil, to terminate by widely expanded free arborizations in the adjacent parts of the pyriform lobe (figs. 22, 23). This connection between the amygdala and the pyriform lobe I call the tractus amygdalo-piriformis, and I believe that it contains, in addition to the

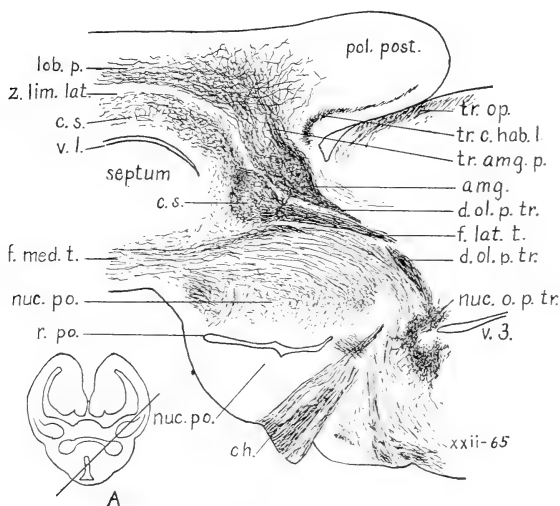


Fig. 23 One section ventrolaterally of figure 22.

fibers just mentioned, axons of the small intrinsic neurons of the amygdala, and fibers passing in the reverse direction from the pyriform to the amygdala, though the last two types of fibers are not clearly demonstrated.

The olfactory projection tracts of the Anura, the diagonal band of Broca, and the stria terminalis may be regarded as parts of a single complex system of correlation fibers which put the lateral olfactory area into physiological relationship with the ven-

tromedial areas of the septum, preoptic nucleus, and hypothalamus. In the frog the lateral connections of this system center in the amygdala, with more or less extensive connections with the overlying pyriform lobe. Its fibers pass partly ventrally of the lateral forebrain bundle and partly dorsally of it.

The fibers of the ventral component which can be followed forward from the amygdala to connect with the nuclei of the septum and rostral end of the preoptic nucleus form the diagonal band. The ventral fibers which are followed spinalward form the ventral projection tract, whose medial connections are unknown, probably chiefly with the caudal end of the preoptic nucleus.

In a similar way the fibers of the dorsal component of this system form a continuous sheet. Those which are followed forward from the region of the amygdala passing above the lateral forebrain bundle to connect with septal areas form the stria terminalis; those which are followed spinalward to connect with the hypothalamus form the dorsal olfactory projection tract. It is probable that both dorsal and ventral components contain correlation fibers passing in both directions between the lateral and medial terminal nuclei throughout their entire extent.

The term olfactory projection tract was first used by Cajal as a synonym for the stria terminalis of lower mammals. The intimate relation of the diagonal band of Broca with this system was recognized by Johnston in the turtle brain ('15, p. 407), and Crosby ('17) described relations of these tracts to the amygdaloid complex in the alligator which are very closely similar to those of the frog. Both of these authors describe in reptiles a component of the amygdaloid complex (medial large-celled nucleus of Johnston, ventromedial nucleus of Crosby) whose topographic relations and fiber connections resemble those of the amygdala of the frog as described above (see beyond, p. 269).

Crosby separated the olfactory projection tract of Cajal into two systems: (1) a system related on the medial side with the preoptic nucleus (and septum?), and (2) a system directed farther caudad to connect with the hypothalamus. To the first she applied the old term stria terminalis; for the second she adopted

Cajal's name, olfactory projection tracts, and she distinguished the dorsal and ventral components of the latter. The relations in the frog are in principle identical with those described in the alligator, and Miss Crosby's nomenclature is here adopted.

The dorsal olfactory projection tract is one of the oldest and most conservative fiber systems in the vertebrate brain, having long been known and described in various types of fishes under the name tractus pallii.

Larval Anura

In half-grown tadpoles of the bullfrog, *Rana catesbiana*, 30 mm. long, horizontal sections show a well-formed vomeronasal formation of the olfactory bulb. The amygdala is clearly recognized as an area of neuropil distinct from that of the striatum. In sections stained with hematoxylin and erythrosin the related cells cannot be separated from those of the striatum and other adjacent parts. The commissure of the amygdalae is large and readily recognized and is quite distinct from the decussation of the lateral forebrain bundles. The other fiber tracts are not revealed by these preparations.

In the brains of much older tadpoles of this species approaching the metamorphosis (150 mm. long), without forelegs and with hind legs 65 mm. long, cut in the horizontal plane and stained with hematoxylin and eosin, the relations of vomeronasal formation and amygdala are essentially as in the adult. The vomeronasal formation is large and very far caudad and is entered by a large and well-isolated vomeronasal nerve. The amygdala is also large and its neurons are clearly separated from those of surrounding regions.

Weigert sections of a slightly younger tadpole of this species, 145 mm. long without fore legs and with hind legs 35 mm. long, show a few myelinated fibers in the tractus amygdalo-habenularis. The 'brown reaction' reveals the course of the ventrolateral olfactory tract clearly and the dorsal olfactory projection tract obscurely.

Our collection contains sections through the entire head of old tadpoles of Pickering's treetoad, *Hyla pickeringii*. These are

about 15 mm. long, with well-developed fore legs, and the metamorphosis was imminent, Dr. McKibben's notes stating, "these toads would have hopped from the water 2 to 4 days after they were killed."

In horizontal sections stained with hematoxylin and eosin the large vomeronasal nerve can readily be followed from the vomeronasal organ back to its termination in the vomeronasal formation, crossing the ventral surface of the main olfactory nerve close to its junction with the olfactory bulb in the way described by McCotter ('17, p. 67) for adult *Rana catesbiana*.

The vomeronasal formation is well differentiated, but is not so large or so sharply separated from the olfactory formation of the bulb as in the tadpoles of *Rana catesbiana* of corresponding age nor are the neurons of the amygdala so clearly separated from those of adjacent areas. In similar sections stained in various ways these relations are confirmed, the ventrolateral olfactory tract can be followed clearly and the dorsal olfactory projection tract obscurely. The nucleus of the latter tract is not well differentiated from the surrounding gray matter in any of these sections of larval forms.

Our material of larval Anura is not extensive; the observations, though fragmentary, are sufficient to indicate that the vomeronasal formation, ventrolateral olfactory tract, and amygdala are organized essentially as in the adult.

III. URODELA

The olfactory nerve of Amblystoma

From our collection of urodeles I shall select for first consideration the brain of adult *Amblystoma*, of which we have abundant material variously prepared. Here we find a very different condition from that described in the preceding section and one that is very instructive from the standpoint of the functional factors which have operated in the morphogenesis of the vertebrate cerebral hemisphere.

In the frog the vomeronasal organ as defined by Gaupp is a small diverticulum from the medial side of the inferior chamber

of the nasal sac. In urodeles the supposed representative of this organ is a lateral pouch from the much simpler nasal sac. In both cases the recess in question is lined with sensory olfactory epithelium. For figures of the nasal organ of *Amblystoma* see Bawden ('94) and Zuckerkandl ('10).

It has long been recognized that in some urodeles there is an accessory olfactory bulb, less clearly separated from the remainder of the bulb than in the frog, which receives a separate posterior and lateral division of the olfactory nerve. In *Amblystoma* Coghill ('02, pp. 209 and 253) demonstrated the course of the posterior division forward, first lying laterally of the anterior division, then ventrally of it, and finally turning abruptly lateralward to connect with the sensory epithelium of the lateral pouch termed Jacobson's organ. He does not exclude the possibility of a certain amount of interchange of fibers between the posterior and anterior divisions, and our Golgi preparations in fact do demonstrate some mingling of these fibers.

I have verified Coghill's description on old larvae of *Amblystoma punctatum* taken a few days before the metamorphosis, save that I have not been able on this material to trace the fibers of the posterolateral division of the olfactory nerve separately from the others of the common trunk for a short distance. These fibers course, as described by Coghill, along the ventral border of the common trunk. Farther rostrally, immediately caudad of the level of the choana, this common trunk breaks up into numerous branches, several of which from the ventral border of the trunk turn laterally along the ventral surface of the nasal sac. One of these supplies the olfactory epithelium of the lateral diverticulum (the supposed vomeronasal organ), others supply the ventral olfactory epithelium medially and caudally of this organ. The peripheral twig which supplies the lateral diverticulum is much smaller than the posterolateral division of the intracranial course of the nerve which terminates in the accessory bulb, and the indications are that this division contains fibers from some of the other peripheral lateral branches referred to above.

Coghill is correct, I have no doubt, in tracing the fibers from the lateral diverticulum of the nasal sac into the accessory bulb, for in well-preserved material this fascicle can probably be readily followed separately; but my material suggests that the incompletely differentiated accessory olfactory bulb receives also many fibers from other parts of the nasal sac.

From these observations and from the accounts in the literature it may be concluded that the nasal sac is much more simply organized in Urodela than in Anura, and that if a true vomeronasal organ is present in the former it is a small lateral diverticulum of the simple sac. In *Amblystoma* the olfactory fibers from this diverticulum terminate centrally in an incompletely differentiated accessory olfactory bulb which also receives other fibers probably derived from the ventral wall of the nasal sac.

These relations strongly suggest the partial homology of the posterolateral division of the olfactory nerve with the anuran vomeronasal nerve and the accessory bulb with the vomeronasal formation; but these names will not be applied to the urodeles in the following description for reasons which will appear in the discussion on pages 255 and 264.

The olfactory bulb and lateral olfactory tract

In adult *Amblystoma* the olfactory bulb is strictly lateral in position (Herrick, '10, figs. 8 to 11; Bindewald, '14, figs. C to F) and occupies the whole thickness of all or part of the lateral wall of the cerebral hemisphere for about half the distance from the rostral end to the level of the interventricular foramen. The caudal end of the bulb (bulbus accessorius of Bindewald, '14, fig. B) is not so clearly separated from the remainder as in the Anura.

The olfactory bulb as a whole forms a considerable eminence on the external surface of the brain and also a projection into the rostral end of the lateral ventricle. The ventricular eminence is in some preparations separated by a slight internal sulcus into larger rostral and smaller caudal portions, thus marking on the ventricular surface the rostral boundary of the accessory bulb. This sulcus is visible only in well-preserved material free from

distortion by shrinkage. The relations of the posterolateral division of the olfactory nerve to the accessory bulb have been described above.

The olfactory bulb on the whole is more simply organized than in the frog. The layer of granule cells is well formed throughout the bulb and in front it curves around the rostral end of the hemisphere to the medial surface. In the latter region there are no mitral cells or glomeruli and the granular layer reaches the external surface (fig. 24). The mitral cells form a compact layer in the rostral part of the bulb, but in the accessory bulb they are more scattered, as in the frog. Nowhere is there developed a definite molecular layer between the granule and the mitral cells.

Except as just noted, the internal structure of the accessory bulb seems to be essentially similar to that of the remainder of the bulb. Mitral cells and subglomerular cells are freely impregnated in our Golgi preparations and the relations of terminals of the posterolateral division of the nerve to glomeruli and mitral cells are similar to those of the anterior division throughout.

From the whole extent of the bulb fibers pass backward into the lateral wall of the cerebral hemisphere as *tractus olfactorius lateralis*, whose dorsal and ventral subdivisions are not so distinct as in the frog. These are mostly unmyelinated, with a few myelinated fibers among them which run for a short distance only (Herrick, '10, p. 422). Some of the myelinated and unmyelinated fibers from the ventral part of the bulb pass dorsocaudad through the deeper layers of the accessory bulb to join the dorsolateral olfactory tract, as in the frog.

The unmyelinated fibers of the dorsolateral olfactory tract are very numerous and form a wide compact superficial fibrous layer over the dorsolateral quadrant of the hemisphere. These secondary olfactory fibers come chiefly from the rostral part of the bulb and are mingled with fibers of the third order (*tractus olfactorialis lateralis*). The dorsolateral quadrant also receives numerous unmyelinated fibers from the accessory bulb which form a zone of open neuropil between the compact dorsolateral tract already mentioned and the *stratum griseum*. These fibers have been mentioned by Bindewald ('14, p. 38).

Unmyelinated fibers from all parts of the olfactory bulb also pass backward in diffuse formation into the ventrolateral part of the hemisphere. These come from both the anterior and posterior parts of the olfactory bulb and pass caudad, partly mingled with but chiefly superficially of the fibers of the lateral forebrain bundle (fig. 25).

Here I must correct the account of the ventrolateral olfactory tract given in my previous description of the brain of *Amblystoma* ('10, p. 422), which was based on material inadequate for accurate study of the unmyelinated fibers. This tract does not arise exclusively from the accessory bulb and pass "directly back close to the ventricular ependyma to end in a cellular thickening at the caudal end of the pars ventro-lateralis opposite the anterior commissure," as in the frog. There is no such specific relation to the accessory bulb or to the amygdala here. Bindewald ('14, p. 39) very correctly failed to confirm my description. The ventrolateral tract occurs, but not as I formerly described it.

Clearly the accessory olfactory bulb is by no means so specific, a structure as in the *Anura*. It is not so well separated anatomically from the rostral part of the bulb and the ventrolateral olfactory tract arises from both of these parts of the bulb and not exclusively from the accessory bulb, as in the frog. This ventrolateral tract, accordingly, is not the exact equivalent of the tract so named in the frog, though the latter is derived from it by the suppression of the fibers from the olfactory formation and further specialization of those from the vomeronasal formation.

The ventrolateral area of the cerebral hemisphere

The forebrain of *Amblystoma* has been several times described, the most important papers being those of Stieda ('75), Herrick ('10), and Bindewald ('14). Figures showing the general structure will be found in the two papers last cited.

The structure of the urodele cerebral hemisphere is much more generalized than that of the *Anura*, though the more important anuran regions can be recognized. The olfactory bulb makes up the entire lateral wall in front and part of it for a considerable

distance farther caudad. The four quadrants into which the remainder of the hemisphere is divided in Anura can be recognized in Urodela; the two medial quadrants are structurally very distinct, but the two lateral are incompletely separated. The lateral forebrain bundle is the chief distinguishing character of the ventrolateral area and the dorsolateral olfactory tract of the dorsolateral area.

In *Amblystoma*, as in the frog, the olfactory bulb extends backward into the ventrolateral area, so that this area does not extend so far forward as does the dorsolateral ('10, figs. 10, 11). The arrangement of cells in the lateral wall of the hemisphere of *Amblystoma* is indicated schematically in figures 8 to 20 of my 1910 paper and in figures A to L of Bindewald's description ('14).

The ventrolateral quadrant is much more simply organized than in the frog. The anuran corpus striatum and amygdala are here completely merged, though the fiber connections of this generalized region show that it contains the primordia of both of these structures.

A thickening of the ventrolateral wall of the hemisphere at the level of the interventricular foramen, less sharply defined than in the frog, contains a considerable collection of nerve cells and a dense neuropil related chiefly to the lateral forebrain bundle and its commissure (figs. 24, 37). This clearly is the urodelean representative of the two separate areas in this region of the frog which have been already described. The diffuse ventrolateral olfactory tract cannot be traced definitely to any part of it, but its fibers are distributed apparently to the entire ventrolateral area, especially its rostral end. This thickening is the only well-differentiated structure in the lateral wall of the urodelean cerebral hemisphere behind the olfactory bulb. Not to prejudice its morphology at the start, it will here be termed the ventrolateral nucleus of the hemisphere.

The rostral end of the ventrolateral area of the hemisphere appears to be physiologically dominated by the secondary olfactory fibers of the ventrolateral olfactory tract, that is, it is a portion of the lateral olfactory nucleus. The caudal end of this

area, the ventrolateral nucleus, is clearly dominated by the lateral forebrain bundle, whose fibers spread freely throughout its stratum album, as well as dorsally into the the overlying dorso-lateral area (figs. 24 to 29, 37). Toward the rostral end of the ventrolateral area these fibers tend to lie deeper than those of the olfactory tract, though the terminals of the two systems are freely mingled.

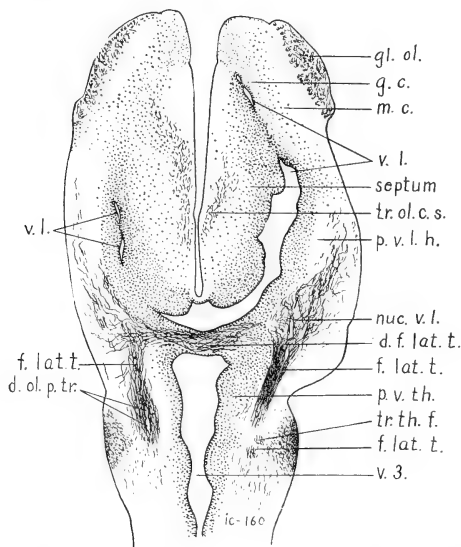


Fig. 24 Horizontal section through the brain of adult *Amblystoma tigrinum*. Weigert method. $\times 17$. The section is inclined so that the left side is farther ventral. It passes through the ventral border of the interventricular foramen and ventrally of the accessory olfactory bulb. On the left side unmyelinated fibers of the dorsal olfactory projection tract (*d. ol. p. tr.*) are demonstrated by the 'brown reaction,' embedded within the lateral forebrain bundle. In neighboring sections these fascicles can be followed forward to their ramification within the ventrolateral nucleus. On the right side the unmyelinated thalamo-frontal tract (*tr. th. f.*) is seen immediately dorsally of its entrance into the lateral forebrain bundle. Its fascicles can be followed forward to ramify within the ventrolateral nucleus, in general dorsally of those of the olfactory projection tract.

The dense neuropil of the ventrolateral nucleus is composed chiefly of terminals and collaterals of fibers of the lateral fore-brain bundle (fig. 28). These are both ascending and descending fibers. Within it are found also the fibers of the dorsal olfactory projection tract, as described below. Myelinated fibers (crossed and direct) connect this neuropil with the habenula in the same way as similar fibers in the frog are related to the amygdala

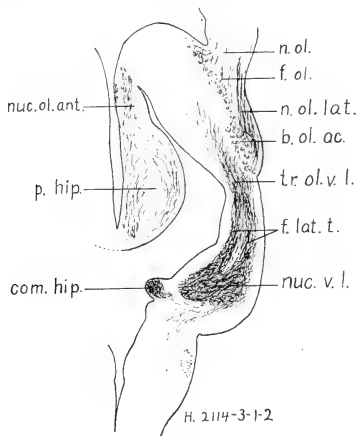
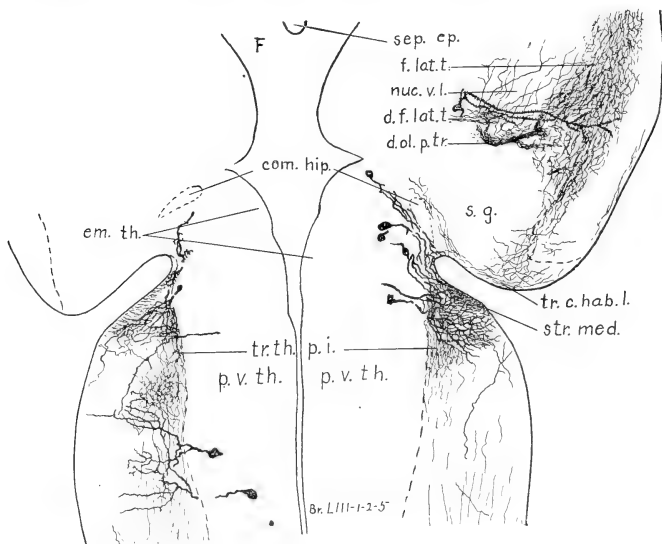


Fig. 25 Horizontal section through the brain of adult *Amblystoma tigrinum* at the level of the interventricular foramen. •Golgi method. $\times 12$. The section illustrates the origin of the ventrolateral olfactory tract from all parts of the olfactory bulb; also the course of the more dorsal fibers of the lateral forebrain bundle and the mingling of these fibers with those of the olfactory tract, in marked contrast with the condition in the Anura, figures 19 and 20.

(tractus amygdalo-habenularis). These are the fibers termed tractus habenulo-striaticus in my earlier paper ('10, figs. 16, 17, 18, *tr. hab. st.*). A large fascicle of the anterior commissure containing both myelinated and unmyelinated fibers connects the two ventrolateral nuclei (figs. 24, 28, and Herrick, '10, fig. 16, *c. a. lat.*). These include decussating fibers of the lateral fore-brain bundles and probably also representatives of the several systems found in the frog connecting the two amygdalae.

The olfactory projection tracts

In urodeles it is probable that there are fiber connections comparable with the stria terminalis, diagonal band of Broca, and ventral olfactory projection tract as described above for Anura; but, if present, these fibers are diffusely mingled with



Figs. 26 to 31 A series of consecutive horizontal sections through the brain of a half-grown larva of *Amblystoma*, arranged in order from dorsal to ventral. Golgi method. $\times 74$. In this series the dorsal olfactory projection tract is impregnated for its entire length and can readily be followed.

Fig. 26 Section through the wide interventricular foramen dorsally of the anterior commissure ridge. Within the ventrolateral nucleus of the cerebral hemisphere some of the more dorsal fibers of the lateral forebrain bundle are impregnated. Among these are slender wisps of the very fine fibers of the dorsal olfactory projection tract which are directed medialward from the tract (cf. fig. 27) to reach the deeper part of the nucleus. Unimpregnated neurons of the stratum griseum fill the medial and caudal parts of the nucleus back to the posterior pole of the hemisphere. Medially of the latter is the eminentia thalami, the axons of whose neurons form a short tract (*tr. th. p. i.*) ending in the pars ventralis thalami, as in *Necturus* (Herrick, '17, p. 291 and fig. 48).

others and they have not been separately followed in our preparations. The dorsal olfactory projection tract, comparable with the tractus pallii of fishes, is, however, clearly defined. The observed relations are indicated diagrammatically in figure 37.

The entire course of the dorsal projection tract is visible in the Golgi preparations from which figures 26 to 31 were drawn. These are horizontal sections through the brain of larval *Amblystoma*, and numerous other preparations by various methods show that the relations of the tract are essentially similar in the adult. These figures are drawn from consecutive sections arranged in order from dorsal to ventral.

Beginning the description at the caudoventral end of the tract and following it forward, we notice that the hypothalamic nucleus of the tract lies relatively farther ventral and caudal than in *Anura*. It is an ill-defined group of neurons (none of which are impregnated in this preparation) lying in the central gray of the hypothalamus at the caudoventral border of the chiasma ridge. Some of its neurons are seen in figure 32. The nucleus is filled with a very dense neuropil derived from several sources and takes the form of a crescent which crosses the midplane in the chiasma ridge, the horns being directed caudad into the hypothalamus (figs. 31 to 34). Within this neuropil are free endings of the fibers of the projection tract, many of which decussate just before terminating.

From the nucleus the tract passes dorsally and slightly laterally across the posterior border of the chiasma ridge, here being surrounded by fibers of the medial forebrain bundle (fig. 30). Upon reaching the level of the lateral forebrain bundle the tract turns abruptly forward embedded within the other fibers of the ventral border of this bundle (figs. 28 to 30), and at the lateral border of the ventrolateral nucleus of the hemisphere its fibers scatter throughout the neuropil of this nucleus (figs. 26, 27).

In none of the urodeles which I have examined, either larval or adult, is the olfactory projection tract related to a special part of the ventrolateral nucleus of the hemisphere, but its fibers are mingled with those of the lateral forebrain bundle in a common neuropil. On the other hand, the tract itself is as large and

clearly defined as in any of the Anura and its hypothalamic nucleus is apparently relatively larger and a more important component of the hypothalamus.

The neurons of this nucleus (fig. 32) are of the same peculiar forms as those which border the infundibulum farther ventrally

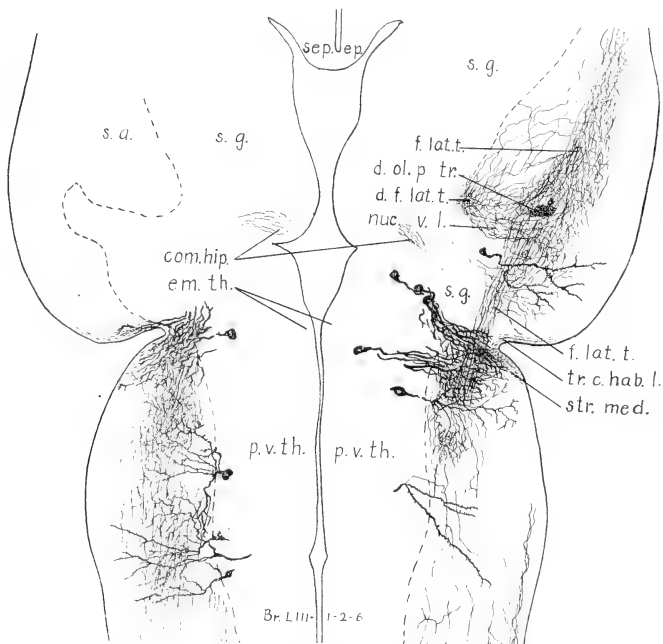


Fig. 27 This section passes immediately dorsally of the anterior commissure ridge. The dorsal olfactory projection tract is a compact fascicle of very fine fibers in the midst of the less densely crowded fibers of the lateral forebrain bundle. As shown by the preceding figure, these fibers turn medialward to enter the deeper part of the ventrolateral nucleus, whereas the greater number of fibers of the lateral forebrain bundle continue forward laterally and dorsally of this neuropil to reach the more rostral region of the ventrolateral part of the hemisphere. Fibers are converging medialward from the neuropil of the ventrolateral nucleus to enter the decussation of the lateral forebrain bundles, most of these fibers being apparently collaterals of those of the forebrain bundle.

and posteriorly. Each is connected with the ventricular surface by a thick process which may join the cell body or the base of the principal dendrite. They are of quite different form from the ependyma cells of this region (fig. 32). These ependyma

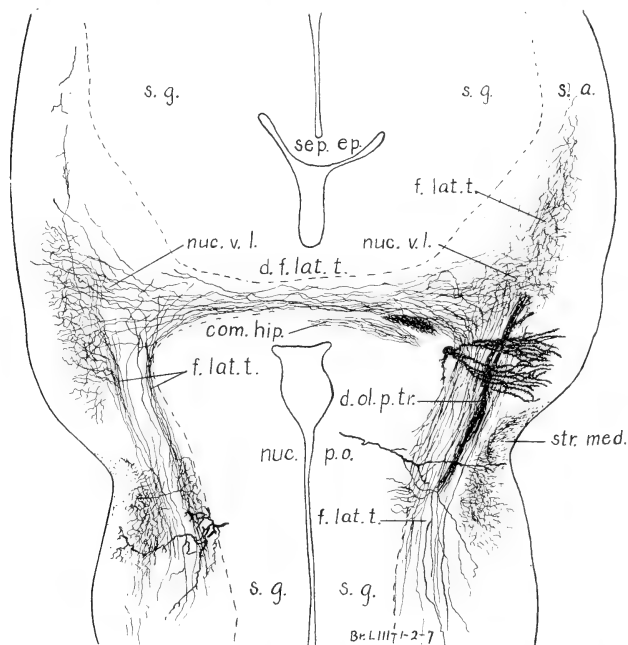


Fig. 28 Section through the dorsal border of the decussation of the lateral forebrain bundles. That segment of the dorsal olfactory projection tract which accompanies the lateral forebrain bundle is visible for its entire length in this section.

cells, moreover, are slender, much branched and varicose, in marked contrast with the mossy ependymal elements found elsewhere in the brains of these larvae. The axons of these neurons, so far as observed, are directed dorsalward and forward

toward the projection tract. The rough and slightly thorny dendrites spread widely forward and lateralward among the terminals of the descending fibers of the projection tract, the medial forebrain bundle, and the most caudoventral component of the postoptic commissure, the tractus thalamo-hypothalamicus cruciatus.

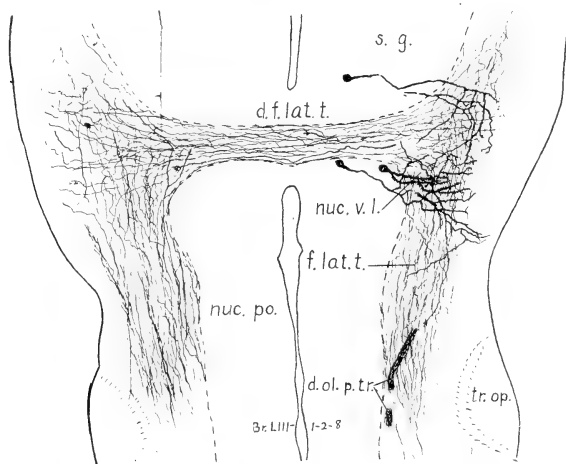


Fig. 29 Section through the ventral part of the decussation of the lateral forebrain bundles. The dorsal olfactory projection tract is turning ventrally and medially to leave the lateral forebrain bundle. The detached portion of the projection tract at the lower end is entered from the adjacent section ventrally, which is otherwise omitted from the series of drawings.

The intimate relationship of the tract last mentioned with this nucleus in *Necturus* I have previously commented upon ('17, pp. 260, 269). In adults and old larvae of *Amblystoma* the same relations prevail (figs. 32, 33). The decussation of the thalamo-hypothalamic tract lies immediately rostrally of the nucleus and is penetrated by dendrites of its neurons. Terminals and collaterals of the tract fibers also turn caudad into the hypothalamus and here engage dendrites of other neurons of the nucleus.

The tractus preopticus of Röthig ('11) reaches the vicinity of this nucleus and may effect functional connections with it. In *Amblystoma* this is a slender strand of a very few myelinated fibers which run longitudinally in the midplane ventrally of the preoptic recess for its entire length. Apparently they are accompanied by a larger number of unmyelinated fibers, but these have not been clearly demonstrated. The myelinated tract

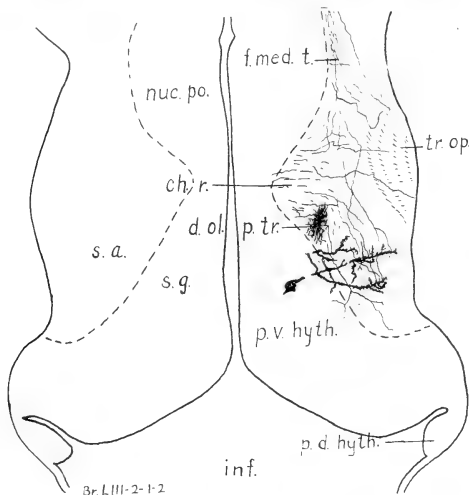


Fig. 30 Section immediately dorsally of the chiasma ridge, showing the olfactory projection tract turning ventrally along its caudal border.

at its caudal end rises up along the rostral aspect of the chiasma ridge and crosses its dorsal surface to disappear at its caudal border. In *Necturus* the fibers take the same course and are clearly traced to a region immediately dorsally of the hypothalamic nucleus of the olfactory projection tract.

Immediately ventrally of the nucleus of the olfactory projection tract in the hypothalamus are neurons of much the same type, whose dendrites probably make the same functional connections as do those of this nucleus, but whose axons appear to be

directed caudad (fig. 33). Many Golgi preparations show that the region reached by these axons is also reached by fibers passing between the wall of the infundibulum and the pars nervosa of the hypophysis, including the saccus vasculosus; but actual continuity of these neurons with the hypophyseal fibers has not been observed. This, however, has been demonstrated by

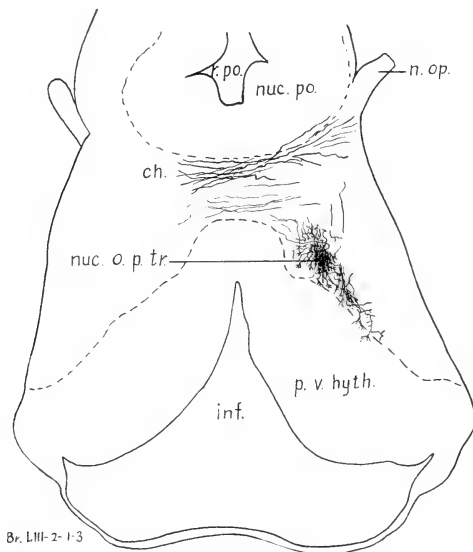


Fig. 31 Section through the nucleus of the olfactory projection tract, showing terminals of fibers of the tract. The more caudal of these terminals are added from the section next ventral to the one drawn.

Bochenek ('02) in *Salamandra*. A similar tract to the saccus vasculosus has been described by others (e.g., Johnston in Acipenser, '01, p. 67), and Cajal ('11, p. 489) describes in the mouse what is probably the same connection, the fibers arising from a cluster of neurons immediately behind the optic chiasma.

Necturus and Amphiuma

In *Necturus maculosus* the relations are broadly similar to those of *Amblystoma*, though there are important differences which have not been fully worked out. There is an accessory olfactory bulb very poorly differentiated from the rostral portion of the bulb. The fibers of the lateral olfactory tract arising from it are distributed to the entire lateral wall of the hemisphere mingled with those from the rostral part of the bulb. There is no separately differentiated amygdala, but a very clearly defined dorsal olfactory projection tract passes between the ventro-lateral nucleus of the hemisphere and the hypothalamus, as in *Amblystoma*.

In *Amphiuma* means the relations are much as in *Necturus*. The large olfactory bulb is fairly uniformly developed in the rostral third of the lateral wall of the cerebral hemisphere. The caudal part of it receives the more lateral bundles of fibers of the olfactory nerve, but there is no clearly defined accessory bulb. Our material does not permit determination of the peripheral relations of the fibers related with the caudal end of the bulb, nor can the unmyelinated fibers of the lateral olfactory tract be analyzed.

The dorsal olfactory projection tract is large and can readily be followed in horizontal sections for its entire course. Its hypothalamic nucleus lies very far ventrally at the caudal border of the chiasma ridge. The fibers of the tract enter the nucleus in many small fascicles, most of which decussate as the most caudal component of the postoptic commissure complex and then spread widely caudolaterad in the deepest part of the stratum album of the infundibulum.

Following the fibers of the olfactory projection tract forward from their nucleus, they are seen to converge into one or several compact fascicles which pass dorsalward along the caudal border of the chiasma ridge, then turn slightly lateralward and abruptly forward embedded among the fibers of the lateral forebrain bundle. Their further course can readily be traced into the neuropil of the ventrolateral nucleus of the cerebral hemisphere,

where they again break up into slender strands which spread out forward and lateralward. No silver preparations of this brain being available, it is impossible to determine the ultimate connections of these fibers. They certainly spread throughout the ventrolateral nucleus and probably reach considerable distances farther forward in the ventrolateral area of the hemisphere and dorsalward into the dorsolateral area and posterior pole.

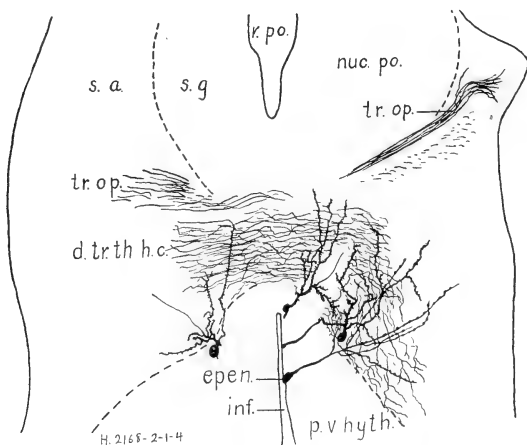


Fig. 32 Three neurons of the nucleus of the dorsal olfactory projection tract of an old larva of *Amblystoma tigrinum*. Drawn from a horizontal section by the method of Golgi. The left side is slightly farther dorsal than the right. $\times 74$. These neurons are in connection with the ependymal surface by thick smooth processes. This process was probably present in the neuron at the left, but is not included in the plane of the section. Many neurons similar to the two at the right are seen in our preparations. Their dendrites are widely branched and somewhat thorny. The smooth axon arises from the base of the dendritic arborization and is directed toward the olfactory projection tract, which it probably enters to ascend to the ventrolateral nucleus of the hemisphere. The dendrites of these neurons ramify in a dense neuropil derived chiefly from the medial forebrain bundle and the tractus thalamo-hypothalamicus cruciatus, the latter only being impregnated in this preparation. This neuropil is greatly simplified in the drawing and is omitted altogether in the regions surrounding the axons of the neurons. A single ependyma cell is impregnated.

IV. MORPHOGENESIS OF THE URODELE STRIO-AMYGDALOID COMPLEX

From the relations described it is evident that the urodeles possess no amygdala as a separate nucleus differentiated from the other elements of the ventrolateral part of the hemisphere. The fiber connections of the ventrolateral nucleus which forms a thickening of the wall in the caudal part of this area show, however, that this nucleus combines the functions of corpus striatum and amygdala, both in a very unspecialized form, that it is, in short, a strio-amygdaloid body.

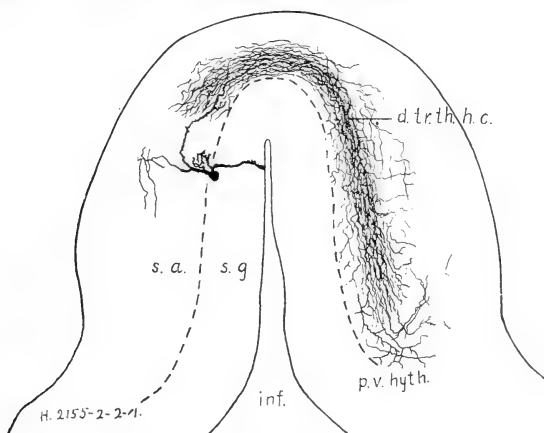


Fig. 33 Horizontal section through the most ventral fibers of the postoptic commissure of an old larva of *Amblystoma tigrinum*. Golgi method. $\times 74$. The single neuron impregnated lies considerably ventrally of those shown in figure 32 and is of different type. As in the other case, the principal dendrite extends forward into the decussation of the tractus thalamo-hypothalamicus cruciatus; but the axon, instead of passing forward into the olfactory projection tract, breaks up into a loose arborization whose longest branches are directed caudad. In other preparations of similar larvae fibers connected with the pars nervosa of the hypophysis extend forward into this position, and this neuron may be the source of such a fiber. It lies ventrally of the nucleus of the olfactory projection tract, though fibers of that tract may extend into this region. Its chief source of excitation is clearly the thalamo-hypothalamic tract, free terminals derived from which reach back almost to the caudal end of the hypothalamus, as shown on the right side of the figure (on the left this neuropil is present, but is omitted from the drawing).

The corpus striatum of the frog is in an exceedingly rudimentary condition, but it is a true striatum (paleostriatum), that is, it is a portion of the lateral wall of the cerebral hemisphere relatively free from olfactory influence. The ventrolateral olfactory tract passes it by to connect with its own specific terminal nucleus farther caudad in the amygdala.

In urodeles, on the other hand, secondary olfactory fibers reach all parts of the lateral wall of the hemisphere, including the

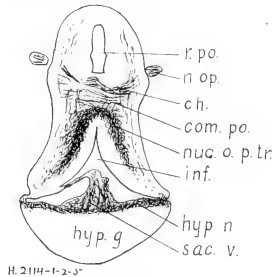


Fig. 34. Horizontal section through the hypothalamus of adult *Amblystoma tigrinum*, from the same specimen as figure 25. Golgi method. $\times 12$. The section passes through the nucleus of the dorsal olfactory projection tract, which is filled with a dense neuropil of fine fibers, which extend caudad through almost the entire length of the pars ventralis hypothalami. The nerve fibers of the saccus vasculosus in the thin roof of the infundibulum and of the pars nervosa of the hypophysis are richly impregnated. Other preparations show that these nerve fibers connect with the hypothalamus, some through the membranous roof of the infundibulum and some through the floor and side walls, and that they extend forward into the region here filled by neuropil from the nucleus of the olfactory projection tract, but mostly farther lateral and caudal.

distribution area of the lateral forebrain bundle in the ventrolateral quadrant. There is, therefore, neither a true corpus striatum nor a true amygdala in the urodele, but a relatively undifferentiated area in the ventrolateral quadrant which retains some of the characteristics of the lateral olfactory nucleus which primitively occupied this region, and at the same time has acquired, under the influence of its ascending non-olfactory connections, the character of a common primordium of both the corpus striatum and amygdala.

The dorsolateral quadrant of the amphibian hemisphere is also a derivative of the primitive lateral olfactory nucleus. In urodeles it is scarcely more than this; in anurans it has assumed more of the characteristics of the mammalian pyriform lobe, but even in mammals this area has deviated far less from the most primitive condition (*viz.*, lateral olfactory nucleus) than has the corpus striatum.

The distinctive characteristics of the differentiated corpus striatum of higher forms include neither olfactory nor hypothalamic connections. Its predominant functional relations are rather with diencephalic sensorimotor systems termed by many recent writers somatic, *viz.*, the somesthetic complex and perhaps the optic and auditory reflex systems. Its chief fiber connections are with the thalamus proper (*pars dorsalis thalami*, as I defined that term in 1910) on the afferent side and with the motor tegmentum of the thalamus and midbrain on the efferent side.

The corpus striatum complex, however, makes its appearance in lower vertebrates within the lateral olfactory area, and there is evidence that, as its phylogenetic differentiation progressed under the influence of increasing numbers of ascending thalamic fibers, the receptive apparatus of the thalamic tracts and the related motor neurons discharging into the tegmentum segregated away from the apparatus receiving the descending secondary olfactory fibers, thus forming the true striatum.

The lateral olfactory area, moreover, in all vertebrates has an important hypothalamic connection comprising both ascending and descending fibers, the tractus pallii of fishes and the olfactory projection tract of mammals. And this hypothalamic connection, unlike the thalamic apparatus, maintains throughout the vertebrate series its physiological relationship with the lateral olfactory centers.

The following physiological factors, accordingly, must be recognized as influencing the morphogenesis of the lateral wall of the vertebrate cerebral hemisphere:

1. The primary influence of the lateral olfactory tract.

2. Ascending thalamic systems (somesthetic, optic, auditory). These came forward originally into the lateral olfactory area, thus effecting various somatic-olfactory correlations. This type of correlation probably survives to some degree in the mammalian pyriform lobe and in part of the amygdala. But the corpus striatum, and in mammals the neopallium, are largely emancipated from the olfactory influence. (The striatum is not entirely so, as shown by Cajal's description ('11, p. 723) of fibers arising

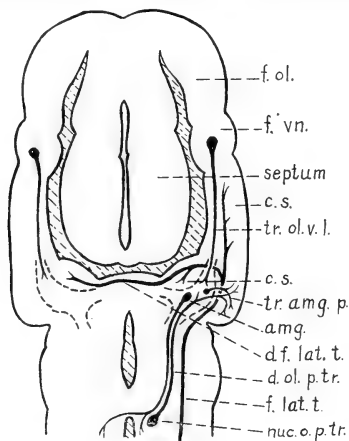


Fig. 35 Diagrammatic horizontal section through the brain of the frog at the level of the interventricular foramen, to illustrate the relations of the ventro-lateral olfactory tract, the dorsal olfactory projection tract, the amygdalo-pyriform tract, and the lateral forebrain bundle.

from neurons of the cortex of the pyriform lobe and entering into the complicated intrinsic neuropil of the lentiform nucleus.)

3. Ascending and descending hypothalamic connections. This is a very primitive connection and apparently originally put the non-somatic systems (as the term somatic is used above) of the hypothalamus into relation with the lateral olfactory area.

4. The habenular connections. These are related to the olfactory centers of the hemisphere only and so far as known are efferent with reference to the hemisphere.

5. Commissural connections.

6. Correlation tracts to and from the medial wall.

In the course of differentiation of the cerebral hemisphere each of these six factors may be variously subdivided and the component parts related physiologically among themselves and to other parts of the brain as required by the mode of life of each species.

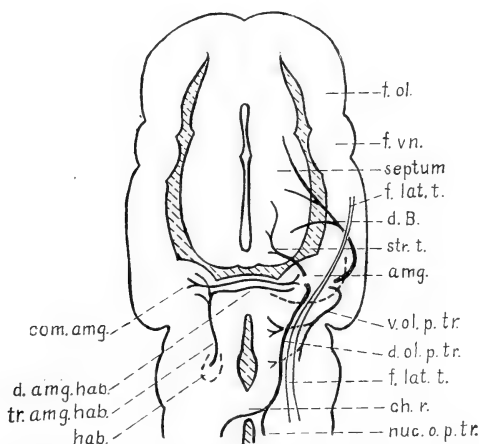


Fig. 36 Diagram of the frog brain similar to the last to illustrate additional connections of the amygdala.

The urodele hemisphere exhibits no regions in the lateral wall behind the olfactory bulb which are related specifically to any of these systems. The anuran dorsolateral and ventrolateral parts of the hemisphere are here incompletely distinguishable only by the internal connections of their dominant fiber tracts. The entire lateral wall, except perhaps the caudal part of the ventrolateral nucleus, receives fibers of the lateral olfactory tracts, and hence is to be regarded as lateral olfactory nucleus, though these fibers are very unevenly distributed within it. Most of the fibers of the lateral olfactory tract enter the compact

dorsal division of the tract, though more diffusely arranged fibers are freely distributed throughout the lateral wall.

The rostral end of the hemisphere is least affected by the ascending diencephalic systems, and it is to this relatively unspecialized part of the olfactory area that the term *nucleus olfactorius anterior* has been applied. In some animals it is an extensive region bordering the olfactory bulb or even extending out into it; in others the elaboration of special centers like the

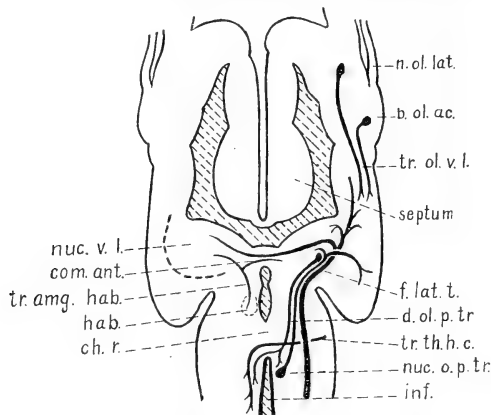


Fig. 37 Diagrammatic horizontal section through the brain of *Amblystoma*, to illustrate the connections of the ventrolateral nucleus of the cerebral hemisphere.

tuberculum olfactorium has invaded this territory so that but little such undifferentiated secondary olfactory tissue remains. In urodeles this anterior nucleus is fairly extensive on the lateral wall, especially dorsally.

The ventrolateral area of the urodele hemisphere is dominated (except at the rostral end) by the lateral forebrain bundle. This contains, in all of the urodele species which I have examined, an extensive system of fine unmyelinated fibers which ascend from the pars dorsalis thalami, which I first called *tractus thalamo-corticalis* ('10, p. 434) and later *tractus thalamo-frontalis* ('17, p. 268).

These fibers unquestionably are the precursors of the mammalian thalamic projection fibers for somesthetic, visual, and auditory sensibility. Within the hemisphere they terminate throughout the ventrolateral area and to some extent at least in the dorsolateral area, especially toward the posterior pole. The return paths for these somatic systems are the strio-thalamic and strio-tegmental fibers of the lateral forebrain bundle. These are myelinated and unmyelinated fibers which arise (so far as now known) from neurons of the ventrolateral area.

The same part of the urodele hemisphere which receives the ventrolateral olfactory tract and the thalamic connections last described is also functionally related with the hypothalamus by the olfactory projection tract (*tractus pallii*), whose ascending fibers also reach the caudal part of the dorsolateral area.

Fibers pass from the lateral wall of the hemisphere to the habenula from the dorsolateral area by the massive *tractus cortico-habenularis lateralis* and from the ventrolateral area by the smaller *tractus amygdalo-habenularis*. The ventrolateral area is connected with the opposite side of the brain through the anterior commissure by decussating and commissural fibers. And, finally, the lateral areas are broadly connected with the medial areas of the hemisphere by extensive systems of association fibers, some of which form the four tracts already mentioned (*diagonal band*, *stria terminalis*, and dorsal and ventral olfactory projection tracts), while others in more diffuse formation cross the dorsal and ventral angles of the hemisphere.

Briefly summarizing our analysis of the lateral wall of the urodele hemisphere, we notice:

1. The olfactory bulb is lateral, with an imperfectly differentiated accessory bulb posteriorly which receives fibers from the supposed vomeronasal organ, as well as fibers from other parts of the nasal sac.
2. The lateral wall of the hemisphere is thin and undifferentiated except for a thickened region laterally of the interventricular foramen, here termed the ventrolateral nucleus of the hemisphere.
3. The entire lateral wall, except perhaps a portion of the ventrolateral nucleus, receives secondary olfactory fibers and

therefore retains the primitive characteristic of the lateral olfactory area. The differentiation within this area is chiefly correlated with the entrance of various specific non-olfactory systems of fibers.

4. The region bordering the olfactory bulb is least affected by these immigrant systems and is termed the nucleus olfactorius anterior.

5. Most of the fibers of the lateral olfactory tract enter the dorsolateral area, which is therefore the chief lateral olfactory nucleus.

6. Posteriorly this quadrant receives ascending thalamic fibers from the lateral forebrain bundle, ascending hypothalamic fibers from the olfactory projection tract, association fibers relating it with the adjacent primordial hippocampus (dorso-medial area), and commissural fibers from the hippocampal commissure; it discharges the lateral cortico-habenular tract to the epithalamus and may also discharge into the hypothalamus. These connections define this region as primordial pyriform lobe (see beyond, p. 271).

7. The relations of the ventrolateral area to the ascending and descending fibers of the lateral forebrain bundle indicate that here is to be sought the functional equivalent of the corpus striatum (paleostriatum); but since there is no group of neurons related with the thalamic and tegmental fiber systems of the lateral forebrain bundle which do not also receive secondary olfactory fibers (by the ventrolateral olfactory tract), or ascending hypothalamic fibers (by the olfactory projection tract), or both of these systems, it is clear that in the urodele there is no anatomical structure to which the name corpus striatum can properly be given.

8. The amygdaloid complex of reptiles (Johnston, '15; Crosby, '17) is related to the lateral olfactory tract, the hypothalamic olfactory projection tract, the stria medullaris for the habenula, the stria terminalis, and the diagonal band of Broca. The first three of these fiber tracts (and probably the other two) are related with the ventrolateral nucleus of the urodele hemisphere, but not with any specific group of neurons within this nucleus. Here

then, is the physiological primordium of the amygdala, but this nucleus as a morphological entity has not emerged from the common olfacto-striatal matrix. In the Anura, on the other hand, there is such a nucleus amygdalae with all of the characteristic connections enumerated above.

V. THE COMPARATIVE ANATOMY OF THE STRIO-AMYGDALOID COMPLEX

Returning now to the Anura, we find the lateral wall of the hemisphere considerably more highly organized. The dorso-lateral quadrant has assumed definite form as lateral olfactory nucleus anteriorly and pyriform lobe posteriorly, much as in lower mammals. It is sharply separated from the underlying ventrolateral quadrant by external and ventricular sulci and between these a histologically defined zona limitans lateralis. The fiber connections are as in urodeles, though more clearly defined.

In the ventrolateral quadrant at the rostral end immediately behind the olfactory bulb there is an undifferentiated region which may receive some secondary olfactory fibers, though our preparations do not reveal them. Behind this is a true corpus striatum, characterized by connection with the lateral forebrain bundle with no olfactory component. This striatal region is enlarged at the level of the interventricular foramen. Closely associated with it, but structurally distinct, is a true amygdala not related to the lateral forebrain bundle, which is characterized by: 1) a specific ventrolateral olfactory tract from the vomeronasal formation of the olfactory bulb; 2) a specific relation with both ascending and descending hypothalamic fibers of the olfactory projection tracts; 3) a habenular connection through the stria medullaris; 4) a dorsal connection with the septum and preoptic nucleus through the stria terminalis; 5) a ventral septal connection through the diagonal band of Broca; 6) a specific commissural connection with the opposite amygdala; 7) a connection with the overlying pyriform lobe through the tractus amygdalo-pyriformis.

The anuran amygdala in emerging from the urodele strio-amygdaloid complex of the ventrolateral nucleus has assumed all of the olfactory, habenular, and hypothalamic connections of the ventrolateral area—connections which, however, it shares with the overlying pyriform lobe. It possesses, moreover, some features which are not shared with the pyriform lobe, viz.:

1. The commissural connection with the opposite amygdala in the anterior commissure. The commissural fibers of the pyriform lobes in mammals pass through the hippocampal commissure, a strictly pallial formation (dorsal psalterium, Cajal, '11, p. 715). In the frog also some fibers of the hippocampal commissure pass through the primordial hippocampus, around the dorsal angle of the hemisphere, to arborize throughout the entire pyriform lobe (P. Ramón y Cajal, '05, pl. 16, fig. 4). I have seen similar fibers from the hippocampal commissure curving around the posterior pole of the hemisphere to reach the pyriform lobe.

2. In the frog the descending fibers of the olfactory projection tract seem to come chiefly (perhaps exclusively) from neurons of the amygdala, while the ascending fibers of this tract terminate freely in both the amygdala and the pyriform lobe. In mammals, on the other hand, Cajal ('11, p. 721) describes the fibers of this tract as arising chiefly as axons of cortical neurons of the pyriform lobe, though he believes that some fibers arise also in the amygdala. The absence of a true cortex in the amphibian pyriform lobe probably accounts for this difference.

3. The secondary olfactory connection of the amygdala is specifically derived from the vomeronasal formation. This point suggests some further reflections.

The absence of the vomeronasal organ in fishes, its very rudimentary condition in urodeles, especially the lower forms, and its close association in many types with the posterior nasal aperture suggest that its primary function is in some way linked with the reception of sensory substances coming from the mouth cavity, though from the relations in higher forms it may be inferred that this primary function has been subject to further modification.

Broman ('20) in a reexamination of the morphological and physiological relationships of the vomeronasal organs of mammals and reptiles has shown that in both of these groups this organ is normally filled with liquid, not air, and that there is a pumping mechanism by which the liquid olfactory medium may be alternately sucked into and expelled from it. This mechanism is very different in the two cases, but in both it appears to be under control and to act rapidly.

In lizards and serpents there is a mechanism by which the liquids of the mouth cavity can be forced into and out of the vomeronasal organ, thus providing "an ideal mouth-smelling organ." In mammals the pumping apparatus is much more complex and diversified in different groups, in some species adapted to draw liquid olfactory media into the vomeronasal organ from the mouth cavity, in others from the nasal cavity, and in most cases from both of these cavities. Broman suggests that the liquid media derived from the respiratory passages of the nose enable Jacobson's organ to function in macrosmatic mammals in 'tracking' by odors (Spürsinn). In this connection it should be borne in mind that the ordinary olfactory epithelium of mammals is not directly excited by gaseous media, as sometimes taught, but the odorous substances must first be dissolved in the liquid which bathes the olfactory membrane. Nevertheless it is not improbable that the liquid of the respiratory passages may absorb a larger amount of the odorous substances and this more concentrated medium, when sucked into the vomeronasal organ, would give to the latter an enhanced olfactory efficiency as a distance receptor.

Broman has not investigated the Amphibia, but from the observations of Bruner already cited (p. 214) it seems very probable that aquatic 'double-smelling' Amphibia developed first a lateral diverticulum of the nasal sac in close relationship with the choana especially adapted to serve as a mouth-smelling organ, and that from this simple beginning the true vomeronasal organs of Anura and Amniota have been derived. Broman ('20, p. 188) himself reached a different conclusion, viz., "that the organon vomeronasale Jacobsoni is nothing other than the old water

olfactory organ of vertebrates adapted for life on land," an untenable position which he unfortunately attempted to support by a reference to the *nervus terminalis* which reveals a total neglect of the recent contributions dealing with the innervation of this region.

The Anura possess a well-developed vomeronasal organ in typical position with a specific vomeronasal nerve related to a specific part of the olfactory bulb, which in turn is connected with the amygdala by a specific olfactory tract. It is strongly suggested that the emergence of a morphologically circumscribed amygdala in Anura is directly correlated with the specificity of its physiological relation with the vomeronasal organ.

All of the fiber-tract connections of the anuran amygdala are probably present in some form in urodeles—certainly the most important ones are clearly recognizable. In urodeles these fiber systems converge into a single non-specific "ventrolateral nucleus." It is not improbable that in the more highly differentiated brains of different types of fishes some of these elements are present but dissociated in various ways. In the Anura the integrating factor which has brought these elements together into a single correlation mechanism and detached this center from the more generalized ventrolateral nucleus of the ancestral form seems to be the vomeronasal organ, for this specific connection is the only obvious physiological factor which is added in the anuran brain.

If, as suggested by Seydel and others (p. 214), the vomeronasal apparatus was differentiated in connection with the opening of the posterior nasal aperture and the consequent passage of olfactory media from the mouth cavity into the nasal sac, it is obvious that from the beginning of this evolutionary process an intimate physiological relationship existed between olfactory excitations of this type and the gustatory excitations arising within the mouth cavity.

Our knowledge of the ascending gustatory path in the brains of vertebrates is very meager. In teleosts it has been shown (Herrick, '05, p. 415) to pass from a reflex correlation center in the isthmus region (the *Rindenknotten* of Mayser, superior secondary gustatory nucleus of later authors) to the region of the

hypothalamus, where its further connections are unknown. In lower Amphibia a similar condition was described (Herrick, '17, p. 248), but the fibers could be traced forward only as far as the level of the III nerve. They probably reach the hypothalamus.²

It is not improbable that there is a direct or indirect connection between the tertiary ascending gustatory system just mentioned and the hypothalamic nucleus of the olfactory projection tract, thus putting the gustatory system into physiological relationship with the olfactory centers of the lateral wall of the cerebral hemisphere. This latter connection is very primitive.³

Now in the Amphibia the opening of the internal nasal passage (choana) introduced a new functional factor into the peripheral olfactory complex, viz., the reception of odorous emanations from the mouth cavity. The central correlation of these newly acquired excitations with those received from taste buds in the course of the feeding reactions naturally accompanied this change in peripheral relations, the mechanism for this being already present in generalized form in the broad connection between the lateral olfactory area and the hypothalamus by way of the olfactory projection tract. This is the condition in urodeles. With the appearance of a vomeronasal organ in definitive form

² In the passage cited ('17, p. 249) I referred to the secondary visceral (and gustatory) nucleus as belonging "in the midbrain, rather than in the medulla oblongata." This statement requires correction; for in view of the fact that the nucleus in question lies behind the sulcus isthmi (as described on page 222 of the same paper), it must be regarded as rhombencephalic if the sulcus isthmi is correctly interpreted as marking the rostral boundary of the rhombencephalon. The corresponding region in teleosts (Ubergangsganglion) has been shown to be rhombencephalic by Palmgren (*Acta Zoologica*, vol. 2, 1921, p. 91).

³ Dart ('20, p. 17) has asserted that the olfactory projection tract (tractus pallii) "is the only ascending tract from the hypothalamus," and that there are no ascending fibers from this region in the medial forebrain bundle. In this he ignores or rejects a considerable body of positive observation, some of it based on ample material stained by the Golgi method, published by competent observers. From the study of my own preparations I have no doubt that there are such ascending fibers in the medial forebrain bundle. The medial wall of the cerebral hemisphere must, accordingly, be recognized as sharing with the lateral wall in the reception of ascending hypothalamic tracts. The broad functional differences between these regions must be interpreted in terms of other factors.

in the Anura there followed differentiation of a specific vomeronasal nerve, vomeronasal formation, ventrolateral olfactory tract, and amygdala, as these are found in the brain of the frog.

In the various fishes the relationships of the structures here under consideration are exceedingly diverse. In ganoids and elasmobranchs the available descriptions do not indicate the presence of any differentiated center which can be compared with the anuran amygdala with any very definite assurance, though in some forms some of the corresponding functional connections are present in a less specialized arrangement more nearly comparable with that seen in the urodeles, and in sharks the differentiation has advanced in a different direction.

In teleosts the nucleus teniae of the carp as described by Sheldon ('12) resembles fairly closely in position and fiber connections the anuran amygdala. It receives fibers of the lateral olfactory tract and sends fibers to the habenula. The tractus pallii (tractus olfacto-hypothalamicus lateralis) reaches the adjacent "nucleus pyriformis," but is not described as entering the nucleus teniae. Similar relations are described in other teleosts by Goldstein ('05), Kappers ('06, p. 11), and others. (The nucleus teniae and tractus teniae of Johnston's descriptions of fishes refer to quite different structures from those so named by the authors just mentioned. Johnston, '11, p. 35.)

The absence of a differentiated vomeronasal organ in fishes raises the question how far these structures can be compared with the anuran amygdala. It is probable that the primordial elements of the amygdalo-pyriform complex of higher brains (and of the hippocampus as well) are here represented in various combinations; but that in none of these species are these elements combined to form an amygdala of the type seen in the frog, for in the absence of the vomeronasal apparatus the integrating factor essential in the latter case is lacking.

Living forms of Amphibia, it is now generally agreed, cannot be regarded as ancestral to any Amniota, so that the anuran type of amygdala is not necessarily the point of departure for this complex as found in higher forms. The elements here associated may be found dissociated or combined in other patterns in

amniote brains. But the specificity of the relationship between vomeronasal organ and amygdala in the frog suggests further lines of interesting inquiry.

In those reptiles and mammals which possess well-developed vomeronasal organs is there a similar specific relationship with any component of the amygdaloid complex?

In the course of amphibian evolution the first component of the amygdaloid complex to emerge in morphologically recognizable form is the anuran amygdala as described in the preceding pages, and this apparently occurred under the specific influence of the vomeronasal system of peripheral connections. In reptiles and mammals, as indicated by Johnston, Crosby, Elliot Smith, and others, other formations from different sources are added to the amygdaloid complex in various patterns. Clearly, then, the structure here designated in the brain of the frog as amygdala cannot be homologized with the whole of the amygdaloid complex of mammals. This has various other elements which may be represented in the urodele ventrolateral and dorso-lateral areas in undifferentiated form.

Johnston ('15, p. 419), in connection with his description of the amygdaloid complex of reptiles, has shown that the mammalian amygdala is a composite of elements of diverse origin. The definition of the mammalian amygdala is, accordingly, very difficult, and in the present state of our knowledge it is of doubtful profit to attempt to fix the mammalian homologies of the anuran amygdala. In reptiles, however, this can be done, as we have seen (p. 236) with some measure of probability (cf. Johnston, '15, p. 418, and Crosby, '17, p. 349), though the absence of the vomeronasal organ in Crocrodilia (Zuckerlandl, '10, p. 35) leaves this question in some obscurity.

The amygdaloid complex of mammals has been the subject of numerous investigations, of which the most extensive is that of Völsch ('06, '10). This author, like Cajal ('11, p. 724), denies direct connection of the olfactory tract with any part of the complex in mammals. This, I think, is very questionable, for my own observations, mentioned below, suggest that the lateral olfactory tract does reach one part of it (the presubicular area),

and such a connection has often been described by others (e.g., Edinger, '11, p. 389.)

In any case, it is clear from Cajal's description that the entire amygdaloid complex of the mouse is under indirect, if not direct, influence from the lateral olfactory tract, for it is enveloped by cortex of the pyriform lobe and is penetrated by axons from these cortical neurons, which in turn receive fibers from the lateral olfactory tract directly. There is also a "tangential tract of the amygdala" (Cajal, '11, p. 725, fig. 463, *d*) between the distribution area of the lateral olfactory tract and the presubicular area of the amygdala.

Röthig ('09), in *Didelphys marsupialis*, describes a nucleus amygdalae under the pyriform lobe and rostrally of this two cellular areas which are probably to be regarded as parts of the amygdaloid complex as this is usually defined in mammals. One of these is a large-celled nucleus occupying the angle ventrally of the pyriform lobe which receives fibers from the lateral olfactory tract; this he terms nucleus of the tractus bulbo-corticalis (our lateral olfactory tract). The second nucleus (his nucleus taeniae semicircularis) lies dorsally and internally of the first and in very close association with it. From it arise fibers of the stria terminalis (his pars ventralis taeniae⁴).

Kappers ('08, p. 241) describes in *Hypsiprymnus* an origin of stria terminalis fibers from a similar nucleus of the lateral olfactory tract and nucleus taeniae. These two nuclei in the aggregate probably correspond pretty closely with the medial large-celled nucleus of Johnston ('15, p. 415) in the turtle and the ventromedial nucleus of Crosby ('17, p. 348) in the alligator.

In rodents the relations are much the same. My observations on the brain of the rat show (in conformity with previous descriptions of rodents) that the most ventral component of the amygdaloid complex is the area presubicularis. This is a sharply circumscribed spherical nucleus of large cells lying close to the surface at the rostral end of the pyriform lobe in much the same

⁴My own observations upon the opossum, *Didelphys virginiana*, suggest that Röthig's account of the relations of the pars ventralis taeniae requires revision, but the discussion of this matter must be reserved until a later time.

relations as in the rabbit (Winkler and Potter, '11). In Weigert sections it appears to receive fibers from the overlying lateral olfactory tract. It gives rise to a fascicle of myelinated fibers of small size which enters the stria terminalis and can be followed separately for the entire length of the stria. They form the most ventral fibers in the dorsal loop of the stria, and many of them can be followed into the anterior commissure. These relations are confirmed in sections stained by Cajal's method. Stria terminalis fibers are related to the entire extent of the amygdaloid complex and the overlying cortex of the pyriform lobe. Some fibers associated with these can be definitely followed into the habenula, but I have not demonstrated that any of these arise specifically from the area presubicularis.

So far as present information justifies conclusions, it may be suggested that the structure here designated amygdala in the Anura is represented in mammals by the presubicular area and the adjacent region termed subiculum cornu Ammonis by Winkler and Potter ('11, fig. xi) and the paleostriatal element of the amygdaloid complex by Dart ('20), though the homology probably is not exact. The remainder of this complex in the Amniota does not seem to be so closely related with the olfactory system and is not represented as a differentiated structure in the frog.

That the olfactory component is not essential to the integrity of the mammalian amygdala is clear from the fact that in the totally anosmic dolphin the amygdaloid complex is of large size, in marked contrast with the atrophied pyriform lobe and hippocampus (Zuckerkandl, '87, p. 113; Addison, '15). The fact that in the dolphin the stria terminalis, though present, is very small (Addison, '15) supports the view that this tract is to be regarded as largely composed in mammals of descending olfactory projection fibers.

The relation of the amygdala to the pyriform lobe requires a further comment. I have earlier ('10, pp. 479, 487) compared the dorsolateral quadrant of the amphibian cerebral hemisphere with the mammalian pyriform lobe, stating, "it is represented in mammals as one of the components of the pyriform lobe," and "the function of the amphibian dorso-lateral part, as of the pyriform

lobe of mammals, is evidently the correlation of olfactory with other exteroceptive impressions belonging to the somatic sensory systems." This conclusion was based chiefly on three anatomical features of this part of the frog brain: 1) it receives the strong dorsolateral olfactory tract; 2) it is broadly connected by correlation fibers with the hypothalamus and primordial hippocampus; 3) it receives ascending non-olfactory fibers from the dorsal part of the thalamus. Kappers (*Folia Neurobiologica*, Bd. 5, 1911, p. 625) questions the validity of the last point on the ground that the mammalian pyriform is not known to receive projection fibers from the dorsal thalamus, and if such are present in the frog their distribution area should be considered neopallial. No such homology as I have suggested can be regarded as exact, for the amphibian brain nowhere exhibits a degree of differentiation which permits clear-cut definition of regions precisely comparable with those of mammals; but the question raises a principle which justifies a further inquiry.

The pyriform lobe of lower mammals is clothed with true cortex, i.e., superficial gray matter, and rostrally this passes by insensible gradations into the lateral olfactory nucleus, a part of the primordial subcortical olfactory area. Immediately internal to this pyriform cortex are the lentiform nucleus and the amygdala. In lower mammals the lentiform nucleus is related by innumerable fibers (probably mostly descending) with the cerebral peduncle, there is a dense neuropil between the lentiform nucleus and the pyriform cortex (Cajal, '11, p. 722, fig. 462), and the nucleus is traversed by very numerous radiating fibers connected with the pyriform cortex. So intimate, indeed, is the connection between the nucleus and the overlying pyriform cortex that Cajal (*loc. cit.*, p. 514) says that the lentiform nucleus seems to be a dependency of the pyriform cortex. This relation is probably reciprocal.

Now in the frog there is no true cerebral cortex in the lateral wall of the hemisphere, but the other structures mentioned are related as just described. And ascending thalamic projection fibers from the thalamo-frontal tract cross the zona limitans lateralis from the ventrolateral quadrant (primordial striatum) to

end in the distribution area of the dorsolateral olfactory tract. It is true that the telencephalic distribution of these thalamic projection fibers in mammals is typically neopallial, and these fibers might be regarded as neopallial in the Amphibia if only there were any neopallium here. The fact is that they reach an area which is dominated by the lateral olfactory tract and which continues to be so dominated even in mammals where it develops true cortex of the pyriform lobe.

The process of the gradual unfolding of the neopallial cortex under the combined influence of functional factors primitively present in the paleostriatum and pyriform lobe has been briefly sketched by Elliot Smith ('19) and need not be reviewed here; but it is interesting to note that during the entire course of this evolutionary history the cortex of the pyriform lobe retains a very primitive character and even in the highest mammals is structurally and physiologically transitional, on the one hand to the subcortical lateral olfactory nucleus and on the other hand to the subcortical corpus striatum and the amygdala. In higher brains the neopallial components of this undifferentiated complex as seen in Amphibia have passed on to more elaborate evolution elsewhere, but the corpus striatum (or part of it) and the pyriform lobe remain as residual structures in practically their primitive relationships.

In this confusing region it is very difficult to determine where are the limits between cortical and subcortical structures. In fact, the terms cortex and pallium are currently employed so loosely that such a determination is impossible without a more satisfactory definition of these terms than has hitherto been published. A clear reformulation of these concepts in the light of recent comparative and embryological studies is urgently needed.

The amygdala of the frog is clearly subpallial in the sense that Gaupp ('99) and I ('10) have defined the pallium; in accordance with the same criteria, the greater part, at least, of the mammalian amygdala appears to be pallial. But careful embryological work will be required before the mammalian complex can be resolved. If I am correct in my belief that the amphibian amygdala

is represented in the mammalian presubicular area (and some contiguous tissue), evidently the name "pallial element of the amygdaloid complex" given to this region by Dart ('20, p. 18) is inappropriate.

The anatomical configuration and connections of the amygdaloid complex suggest that as a whole it possesses a certain physiological unity and that the component parts of this integrated complex are diversely represented in various vertebrate types, in accordance with their respective modes of life. The amphibian relations suggest, further, that the correlation of olfactory, gustatory, and perhaps other excitations arising from food within the mouth was the original integrating physiological factor. The complex of higher forms probably includes also connections of the tactual and muscle senses.⁵ Though the differentiation of the vomeronasal organ peripherally was the initial point of departure for the fabrication of the amygdala, the complex, once developed, retains its individuality in the absence of the vomeronasal organ (alligator, man), and even of the entire olfactory system (dolphin).

The mammalian amygdala, like many other complex correlation centers, is a mechanism in which there converge into final common paths numerous very diverse kinds of peripheral excitation—some of visceral (interoceptive) type and some of somatic (exteroceptive) type. In some species one of these types may be dominant, in other species the other, and the analysis of these components must be carefully worked out in each vertebrate group before we can generalize profitably. In the Amphibia the visceral components are clearly predominant, and this appears to be the primitive situation.

The vigorous and rather temerarious attack of Dart ('20, p. 18) upon this method of cerebral analysis is evidently based

⁵ The tactual factor may in some animals be very important. In this connection it is interesting to note that Broman ('20, p. 178) considers that the forked tongue of serpents is accessory to the vomeronasal organs which here are very large, the extruded tongue taking up odors and carrying them back to the openings in the roof of the mouth of the ducts of these organs, which indeed the tips of the tongue may enter. The serpent's tongue is generally regarded as a very efficient tactile organ, and this function also, accordingly, would naturally be largely represented in the amygdaloid complex of serpents.

upon an inadequate appreciation of the underlying physiological principles involved. It is generally recognized that the analysis of the vertebrate nervous system in terms of the physiological modalities of the related peripheral end-organs has greatly clarified an obscure field. The detailed application of this method of attack is the distinctive contribution of the so-called American school, beginning with an adequate analysis of the functional components of the peripheral nerves and carrying this analysis more and more deeply into the intricacies of the higher correlation centers.

The first generalizations to be derived from these studies (Strong, '95) brought out in sharp relief the fundamental nature of the distinction drawn by some of the early English physiologists, notably Bell and Gaskell, between the somatic and visceral systems of nerves, a conception later employed with brilliant results by Sherrington.

This method of analysis has clarified the central nervous system as helpfully as the peripheral, and its value in the forebrain is not less than in the medulla oblongata. In fact, the tardy progress in the comparative morphology of the forebrain has been due chiefly to lack of exact knowledge in just this field, to ignorance of just what peripheral systems are represented in the higher correlation centers of the various animal types under investigation. Before we can reach final conclusions regarding the origin of the cerebral cortex we require much more detailed information regarding the connections of the pre-existing subcortical mechanisms of the forebrains of critical types. This applies especially to the centers of correlation between olfactory and non-olfactory systems.

What names are applied to these systems is a relatively unimportant matter, but it is necessary to bear in mind that the olfactory system itself is a complex in which visceral (interoceptive) and somatic (exteroceptive) elements are always present. To deny or ignore this dual nature of the sense of smell is to close the door to further progress.

The olfactory apparatus, whatever may have been its primitive physiological character, has in fishes (as in most higher verte-

brates) come to be one of the dominant exteroceptive systems, that is, it functions as a distance receptor. Nevertheless, throughout the entire phylogenetic history of this system it exhibits pronounced interoceptive or visceral functions associated with the selection of food, functions closely similar to those of the gustatory system (Herrick, '08). In man, a microsmatic animal, the sense of taste and the interoceptive aspects of the sense of smell are so intimately related that we are unable introspectively to separate them, and physiological experimentation is required to effect this analysis. One wonders whether, if we were provided with a functional vomeronasal organ and its specific central apparatus as this is seen in the frog, our sensory experience would not thereby be enlarged.

VI. SUMMARY

1. The vomeronasal organ (Jacobson's organ) first appears in Amphibia, apparently in correlation with the opening of the posterior nasal aperture. In Urodela it is present (if at all) in very rudimentary condition, but in Anura it has assumed definitive form as a diverticulum from the medial side of the nasal sac provided with its specific innervation, the vomeronasal nerve.

2. The vomeronasal nerve terminates in a specific part of the olfactory bulb, the vomeronasal formation, and from the latter a specific secondary path, the ventrolateral olfactory tract, passes to a differentiated amygdala in the ventrolateral wall of the cerebral hemisphere.

3. The other connections of the anuran amygdala are: with the opposite amygdala through the anterior commissure; with the adjacent pyriform lobe; with the medial olfactory areas (septum and preoptic nucleus); with the habenula; and with the hypothalamus. The connection last mentioned is by descending and ascending fibers of the olfactory projection tract, which is the equivalent of the tractus pallii of fishes.

4. The frog possesses a true corpus striatum (paleostriatum) specifically related with the lateral forebrain bundle and quite separate from both the lateral olfactory nucleus and the amygdala.

5. In some urodeles the supposed primordium of the vomeronasal organ sends nerve fibers to the accessory olfactory bulb, which also receives fibers from other parts of the nasal sac and is therefore not the exact equivalent of the vomeronasal formation of the frog. There is a ventrolateral olfactory tract whose fibers arise from the accessory olfactory bulb and also from other parts of the bulb and, accordingly, are not specifically related to the vomeronasal organ, as in the frog.

6. The ventrolateral area of the urodele cerebral hemisphere comprises an undifferentiated strio-amygdaloid primordium in which the fiber connections described above as characterizing the corpus striatum and amygdala, respectively, of *Anura* are inextricably mingled. This is termed the ventrolateral nucleus of the hemisphere. The hypothalamic connection of this nucleus (olfactory projection tract) is large and well defined.

7. In both *Urodela* and *Anura* the hypothalamic nucleus of the olfactory projection tract is in functional connection with the septal areas through the medial forebrain bundle, with the dorsal part of the thalamus through the tractus thalamo-hypothalamicus cruciatus, probably with gustatory and other visceral systems of the hypothalamus, and possibly with the pars nervosa of the hypophysis.

8. The lateral wall of the amphibian cerebral hemisphere is derived from the lateral olfactory area of more primitive vertebrates. The course of differentiation of this region has been largely determined by the penetration into it of ascending diencephalic fibers of two systems: 1) somatic fibers from the dorsal part of the thalamus through the thalamic projection tracts, and 2) visceral fibers from the hypothalamus through the olfactory projection tracts. The first factor has dominated the further differentiation of the striatal and neopallial complexes; the second that of the amygdala and pyriform-lobe complexes.

9. The olfactory system in all vertebrates has a twofold functional rôle: 1) visceral or interoceptive in relation with the selection and digestion of food, and 2) somatic or exteroceptive in relation with the adjustment of the organism to environmental conditions. With the opening of the posterior nasal aperture

in the Amphibia, the vomeronasal organ appears to have been differentiated in connection with the first of these factors, namely, for cooperation with taste and perhaps various tactual and other general forms of sensibility within the mouth, in the analysis and appropriate disposition of the contents of the oral cavity.

10. The anuran amygdala was apparently differentiated under the influence of the specific sensory excitations coming from the vomeronasal organ. So far as the pre-existing nervous pathways connected with the ventrolateral area of the cerebral hemisphere conducted excitations physiologically congruous with those from the vomeronasal organ, these were integrated into a morphological unity as we see it in the amygdala of the frog.

11. The amygdaloid complex having been thus integrated under the dynamic influence of vomeronasal excitations, further complication of the system progressed in higher vertebrates in various directions by the incorporation of other related sensory systems, with perhaps profound modification of the functional aspect of the complex as a whole. So great may be this deviation from the primitive physiological pattern in some cases that the suppression of the vomeronasal organ, as in man, or even of the entire olfactory system, as in the dolphin, does not destroy the integrity of the surviving components of the amygdaloid complex, which retains its individuality in modified form.

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Resumen por el autor, Chi Ping.

Sobre el crecimiento de las mayores células nerviosas del ganglio superior cervical del simpático de la rata albina desde el nacimiento hasta la edad adulta.

Las mayores células nerviosas de este ganglio presentan un rápido crecimiento durante los primeros veinticinco días después del nacimiento, seguido de una fase de crecimiento más lento. Después de la pubertad existe una tendencia en la hembra a presentar células mayores que las del macho de la misma edad. El crecimiento de estas células está más intimamente relacionado con la edad que con el peso del individuo. Unas pocas células en fases avanzadas se presentan al tiempo del nacimiento; su número aumenta lentamente antes del décimoquinto día y después crece más rápidamente. Relacionado con esto hay una disminución en el número de pequeñas células que se transforman en células mayores. Estas pequeñas células parecen transformarse en mayores números en la hembra durante la edad más avanzada. La relación nucleo-plasmática aumenta desde 1 hasta 4 en la época del nacimiento, hasta llegar a ser aproximadamente 1 a 12 durante la edad adulta. En la edad adulta las células grandes pueden distribuirse en tres grupos según la distribución de sus granos de Nissl. Existen unas cuantas células binucleadas durante todas las edades y después de la pubertad también hay células pigmentadas. Las observaciones anteriores se refieren a ratas albinas *standard*. En los albinos cruzados entre sí (*inbred*) existen siempre células más pequeñas, con una pequeña diferencia sexual en el tamaño de las células y una relación nucleo-plasmática menor. El autor no explica esta diferencia entre las dos castas de ratas albinas.

Translation by José F. Nonidez
Cornell Medical College, New York

ON THE GROWTH OF THE LARGEST NERVE CELLS IN THE SUPERIOR CERVICAL SYMPATHETIC GAN- GLION OF THE ALBINO RAT—FROM BIRTH TO MATURITY

CHI PING

The Wistar Institute of Anatomy

SIX CHARTS AND ONE PLATE

INTRODUCTION

This paper contains observations on the largest nerve cells in the superior cervical sympathetic ganglion of the albino rat. The purpose of this study is to trace the growth of these cells by their increase in diameter in relation to the age and size of the animal. In order to compare the possible differences in growth in the sympathetic nerve cells due to sex, a male and a female rat of each age were used throughout the series of observations.

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MATERIAL

The material used for this investigation consisted of sixteen pairs of albino rats, of known ages, from 1 to 365 days. Besides these, two females of 540 days and 570 days, respectively, were used for comparison. All these were obtained from the animal colony at The Wistar Institute and belonged to the so-called 'standard strain.' In selecting the specimens, five-day intervals were taken between each two ages from birth to thirty days, but from this age onward greater intervals were used. The body weight, body length, sex, and age of each rat were recorded.

For comparison and control a second limited series of inbred albino rats was also used. The data for this series are given on page 303. Up to the introduction of this series the paper deals only with albino rats of the 'standard' strain.

TECHNIQUE

The rat was etherized and, after the necessary measurements had been noted, was completely eviscerated. The superior cervical sympathetic ganglion was removed from each side. In the removal care was exercised to avoid distortion of the tissue, for mechanical injury to the ganglion is likely to affect the size and shape of its cells. As the ganglion is small, it was deemed necessary to remove it in the mass of other tissues which closely invest it.

Both ganglia from each rat were prepared, but only one was used for measurements. No distinction between right and left was made in the record.

Aiming at a satisfactory preservation of the natural size of the cells, I followed King's ('10) recommendation of Bouin's solution for fixation. The ganglia from older rats were fixed in the solution for twenty-four hours, while for those from the younger ones—from birth to twenty-five days old—the period was reduced to twelve hours. Such a reduction of the fixation period has given satisfactory results.

The specimen was washed in different grades of alcohol, from 70 to 98 per cent, containing a small amount of carbonate of lithium. By so doing the yellow tinge given to the tissue by the fixation was completely removed. The specimen stayed in the alcohols of lower grades for twelve or more hours, and in the 90 and 98 per cent alcohol for about one hour. It was finally transferred to cedar oil for twenty-four hours for complete dehydration. Paraffin of 52° was used for imbedding. By employing an electric bulb above the container the paraffin was kept melted only in its upper layer in the jar, the specimen sinking to the contact line between the melted and unmelted paraffin.

Under these conditions the specimen could be left in the paraffin for thorough penetration as long as seemed necessary without danger of overheating.

Serial sections of the entire ganglion were cut $8\ \mu$ in thickness. Heat from an electric bulb was used in flattening the sections. The slide was placed underneath the bulb, so that the water that served to float the sections on the slide, also chilled them from beneath, when they were spread by the heat.

The procedure in staining was as follows: The sections were passed from xylol down through the graded alcohols to water, and then put for five minutes in a saturated solution of lithium carbonate, after which they were stained for two or three minutes in a one-third saturated solution of thionin. They were then passed up through the graded alcohols to xylol and mounted in acid-free balsam. So far as possible, the plane of section was made perpendicular to the short axis of the ganglion, thus giving the maximum area.

MEASUREMENTS OF THE CELLS AND NUCLEI

The cells and nuclei of the ganglion were measured with an eyepiece micrometer, using a Zeiss ocular no. 6, and objective, 4 mm. Each division in the micrometer scale was equivalent to $4.47\ \mu$. The measurements were made in the following way: In the case of each specimen a section at the middle of the series was selected. Starting both ways from this, four more sections were selected, two in each direction, by skipping every other section. In this manner five sections altogether were chosen and marked for study. In each of the five sections the two largest cells were measured; thus ten cells in all were measured in each ganglion.

There were four principal points kept in mind when selecting the cells for study: First, the cells must be the largest in the section; second, they must be uninuclear; third, the nucleus must be located at or near the center of the cell and must be fairly large; fourth, in the nucleus at least one nucleolus must be present.

Under these conditions, the measurements made on the cells and nuclei are considered to represent the maximal longitudinal and transverse diameters of each cell and nucleus taken close to their median planes. It was often found in this study that

the boundaries of a cell body were obscure. Furthermore, the distribution of the Nissl granules was rather irregular (as will be described later), so that neither the longitudinal nor the transverse diameter could be measured according to the extent of the stainable mass.

After the measurements had been taken, a sketch of the section with the two cells measured therein was made, and the nucleoli in these cells were noted, so that in making measurements for the second time the same cells could be identified by their location and the number of the nucleoli. As a matter of routine, the cells in each ganglion were measured twice, a considerable time being allowed to elapse between the first and second measurements. The procedure in measuring did not follow in the order of age or of body weight of the animal, as given in the tables, but was purposely haphazard, and in making measurements for the second time, the records were taken without referring to those already made. The values used are the means of the two series.

By this procedure prejudice was avoided and a more accurate determination of the size of the cells and nuclei obtained. The records thus made were tabulated in detail, but the averages of the values for the ten cells in each ganglion are those used for the tables, charts, and discussion which follow. The individual data have been filed in the archives of The Wistar Institute.

The square roots of the products of the longitudinal and transverse diameters of the cells and of the nuclei, respectively, for each ganglion were averaged, and the mean was multiplied by 4.47, the value in μ of one division of the eyepiece scale. In table 1 the diameters of the cells and nuclei thus computed are arranged according to age, and in table 2 according to the body weight of the animal.

Based on the records in tables 1 and 2, charts 1 and 2 were plotted. Chart 1 shows on age the graphs for the diameter of the cells and nuclei in micra and chart 2 the same relations on body weight.

In the graphs for the cells in chart 1 we see in the increase before puberty only chance variations between the male and the female in diameter of the cell body, but after the rat has attained

the age of eighty days (body weight about 100 grams) which is the period of puberty (Donaldson, '15, *The Rat*, p. 21) there appears a tendency for the cells to be larger in the female than in the male. It will be noted, however, that at the age of eighty-nine days, and also at 250 days, the male exhibits larger cells than the female of the same age. This discrepancy is explained when we take the body weights of the males into consideration. As given in table 1, the body weight of the male rat eighty-nine days old is twice that of the female of the same age, and the discrepancy is even greater in the case of the male at 250 days.

These males should be expected to have larger nerve cells by virtue of their body weight, and when a correction is made for it, the values for the male cells should fall below those for the female at these ages also. In general one may say that the female, after reaching puberty, has these cells larger than the male, if the body weight of the male does not too greatly exceed that of the female. As regards the nucleus, however, chart 1 exhibits a less clearly marked sex difference.

The fact that there is a better growth of the cell bodies in the female is more clearly illustrated in chart 2, in which graphs for the diameters of the cells and nuclei have been plotted on body weight. From birth to the time just before puberty, the variations in the growth of these cells in the two sexes are similar to those shown in chart 1.

Just before puberty, when the rat weighs about 60 grams, the female becomes gradually more advanced in the growth of these cells and overtakes the male of the same body weight. The growth of the nerve cells in the female also shows a more regular course than that of the male. The growth of the nuclei at the corresponding ages of the two sexes follows in the same manner as that of the cell bodies, although the difference is relatively small.

It is proper to keep in mind, however, that when the comparison is made on the basis of body weight, the female is normally the older, and, further, that in several other growth changes the female tends to be precocious; both of these influences would tend to produce larger cells in the female under these conditions.

TABLE 1

Computed diameters of the largest cells and nuclei according to age. From the superior cervical sympathetic ganglion of the albino rat

SEX	A	B	C	D	E	F
	AGE	BODY WEIGHT	BODY LENGTH	Computed diameter in μ		RATIO OF DIAMETER OF NUCLEUS TO DIAMETER OF CELL
				Cell	Nucleus	
	<i>days</i>	<i>grams</i>	<i>mm.</i>			
♂	1	5.6	50	19.5	11.4	1:1.72
♀	1	6.3	51	19.8	10.2	1:1.93
♂	5	9.0	63	22.1	10.7	1:2.06
♀	5	11.0	65	21.3	10.5	1:2.03
♂	11	15.0	77	24.9	13.1	1:1.90
♀	11	14.0	73	26.4	13.1	1:2.02
♂	16	18.9	83	25.3	13.1	1:1.92
♀	16	19.0	81	23.1	11.2	1:2.06
♂	20	31.7	102	26.4	12.5	1:2.11
♀	20	29.5	99	23.6	11.8	1:1.99
♂	25	23.8	93	26.6	12.6	1:2.11
♀	25	25.5	95	27.3	12.7	1:2.15
♂	29	40.7	112	27.1	12.0	1:2.26
♀	29	16.4	82	24.8	12.2	1:2.03
♂	42	61.4	129	27.0	13.4	1:2.01
♀	42	43.5	105	27.2	13.2	1:2.05
♂	48	105.1	156	29.0	13.4	1:2.17
♀	48	49.7	120	27.0	13.1	1:2.05
♂	60	51.6	124	27.2	13.2	1:2.06
♀	62	53.8	117	27.1	13.1	1:2.07
♂	81	63.3	128	27.4	13.3	1:2.06
♀	80	83.7	142	26.6	12.8	1:2.09
♂	89	143.5	173	32.4	13.0	1:2.49
♀	88	73.0	135	29.2	13.2	1:2.21
♂	124	151.1	174	27.1	13.0	1:2.08
♀	124	107.1	157	30.5	13.8	1:2.21
♂	171	198.2	192	27.0	13.1	1:2.05
♀	171	123.8	159	30.9	12.8	1:2.41
♂	250	230.0	207	36.8	15.4	1:2.38
♀	250	98.0	160	30.6	14.2	1:2.15
♂	365	186.0	203	29.6	13.5	1:2.20
♀	365	170.6	186	31.4	13.5	1:2.31
♀	540	151.3	184	30.7	13.4	1:2.29
♀	570	127.1	169	33.4	14.3	1:2.34

On examining the ratios between the values at one day and at 365 days, as shown in columns D and E of table 1, it is found that the cells in the male have increased in diameter 1.55 times, and in the female 1.58 times, while the nuclei in the male have increased 1.17 times and in the female 1.32 times. This shows that the difference between the male and female in the growth of the nuclei in the course of one year is greater than that in growth of the cells, but the cells in both sexes have a greater rate of growth than do the nuclei, as indicated in table 1.

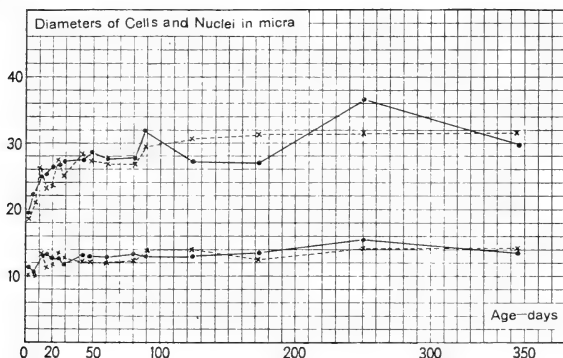


Chart 1. Based on table 1 and giving the computed diameters of the cells and their nuclei according to sex—on age in days. Males ————— Females - - - - -

The graphs in chart 1 show that the increase in the diameter of the cell body is rapid for the first twenty-five days and then becomes slower. There is a similar change in the nucleus, though the change in the rate of growth in this case is less marked. From 25 to 365 days the diameters of the cells and of the nuclei of the two sexes have increased as shown in table 3.

In column F of table 1 are given the ratios between the diameter of the cell body and that of the nucleus. Generally speaking, the cell has about twice the diameter of the nucleus throughout the series of measurements as given in table 1, but if we consider the ratios carefully, we see that there is an increase in the ratios

TABLE 2

Computed diameters of the largest cells and nuclei—on body weight—together with the nucleus plasma ratios—from the superior cervical sympathetic ganglion of the albino rat

SEX	A	B	C	D		E	F
	AGE	BODY WEIGHT	BODY LENGTH	Computed diameter in μ		NUCLEUS PLASMA RATIOS	
				Cell	Nucleus		
	<i>days</i>	<i>grams</i>	<i>mm.</i>				
♂	1	5.6	50	19.5	11.4	1: 4.0	
♀	1	6.3	51	19.8	10.2	1: 6.3	
♂	5	9.0	63	22.1	10.7	1: 7.8	
♀	5	11.0	65	21.3	10.5	1: 7.3	
♀	11	14.0	73	26.4	13.1	1: 7.2	
♂	11	15.0	77	24.9	13.1	1: 5.8	
♀	29	16.4	82	24.8	12.2	1: 6.8	
♂	16	18.9	83	25.3	13.1	1: 6.2	
♀	16	19.0	81	23.1	11.2	1: 7.7	
♂	25	23.8	93	26.6	12.6	1: 8.4	
♀	25	25.5	95	27.3	12.7	1: 8.9	
♀	20	29.5	99	23.6	11.8	1: 7.0	
♂	20	31.7	102	26.4	12.5	1: 8.0	
♂	29	40.7	112	27.1	12.0	1:10.5	
♀	42	43.5	105	27.2	13.2	1: 7.7	
♀	48	49.7	120	27.0	13.1	1: 7.7	
♂	60	51.6	124	27.2	13.2	1: 7.7	
♀	62	53.8	117	27.1	13.1	1: 7.8	
♂	42	61.4	129	27.0	13.4	1: 7.2	
♂	81	63.3	128	27.4	13.3	1: 7.5	
♀	88	73.0	135	29.2	13.2	1: 9.8	
♀	80	83.7	142	26.6	12.8	1: 7.9	
♀	250	98.0	160	30.6	14.2	1: 9.0	
♂	48	105.1	156	29.0	13.4	1: 9.1	
♀	124	107.1	157	30.5	13.8	1: 9.8	
♀	171	123.8	159	30.9	12.8	1:13.1	
♀	570	127.1	169	33.4	14.3	1:11.7	
♂	89	143.5	173	32.4	13.0	1:14.4	
♂	124	151.1	174	27.1	13.0	1: 8.0	
♀	540	151.3	184	30.7	13.4	1:11.0	
♀	365	170.6	186	31.4	13.5	1:11.6	
♂	365	186.0	203	29.6	13.5	1: 9.5	
♂	171	198.2	192	27.0	13.1	1: 7.7	
♂	250	230.0	207	36.8	15.4	1:12.6	

as age advances, as they are, respectively, 1 : 1.72 and 1 : 1.93 for the youngest male and female; 1 : 2.11 and 1 : 2.15 at twenty-five days, and 1 : 2.34 for the oldest female.

This increase is therefore most marked during the first twenty-five days. By comparing the progress from birth to twenty-five days with that from twenty-nine days to 365 days, one can appreciate the rapid increase in amount of cytoplasm within the former period, as contrasted with the slower increase in the course

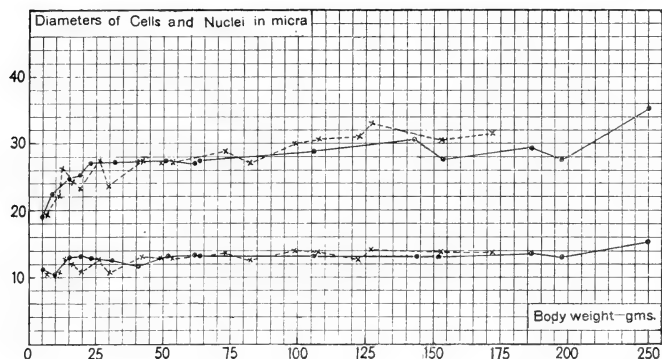


Chart 2 Based on table 2 and giving the computed diameters of the cells and their nuclei according to sex—on body weight. Males ————— Females - - - - -

of a much longer time. It is fair to say, therefore, that the ratios change but slowly after the first twenty-five days. This agrees with the statement of Donaldson and Nagasaka ('18) on the ventral horn cells, that after twenty-four days the nucleus-plasma ratio increases but slowly.

VARIABILITY WITHIN A GIVEN GANGLION

The number of large cells examined in each ganglion is hardly great enough to permit of satisfactory statistical treatment, but it has been thought worth while to tabulate for each animal the range and average of the diameters of the cells and of the

nuclei, entering these according to age as in table 1. In a fairly graded series of measurements we may expect to find the average for the series close to the mean of the limiting values, and a little study of table 4 shows this to be the case.

MORPHOLOGY OF THE LARGE CELLS

Plate 1. (Figures 1 to 7)

In considering the morphology of the cells in the superior cervical sympathetic ganglion, it must be recalled that from its cells arise several classes of fibers—pupillodilator fibers, motor, vasomotor, pilomotor and secretory fibers. It is a priori possible,

TABLE 3
Increase in diameters of cells and nuclei from 25 to 365 days

DIAMETERS	SEX	25 DAYS	365 DAYS	GAIN	
				Absolute	Percentage
		μ	μ	μ	
Cells.....	♂	26.59	29.60	3.01	11.3
	♀	27.26	31.38	4.12	15.1
Nuclei.....	♂	12.60	13.45	0.85	6.31
	♀	12.65	13.54	0.89	6.58

that the several functions thus indicated are correlated with cell characters that are distinctive, but at the moment we have nothing to contribute to the solution of this problem.

When young, the cells of the superior cervical ganglion are very similar in appearance to those of the young spinal ganglion, and practically all of them are more or less elongated with processes at one or both ends. Each cell has a large clear nucleus surrounded by a little cytoplasm. This cytoplasm is homogeneous in structure and stains uniformly. Those coarse Nissl bodies, which are found in the cells at later ages, are totally lacking. Usually each nucleus has a single, dark stained, nucleolus, but occasionally there may be found more than one. This condition continues from birth to five or six days of age, when differentiation begins in the cytoplasm of these immature cells.

TABLE 4

*Giving the ranges in the values for the diameters of the largest cells and their nuclei in the superior cervical sympathetic ganglion of the albino rat—
arranged according to sex and age*

SEX	AGE	BODY WEIGHT	CELLS		NUCLEI	
			Diameter	Range	Diameter	Range
	<i>days</i>	<i>grams</i>	μ	μ	μ	μ
♂	1	5.6	19.5	22.0-18.0	11.4	14.0- 9.4
♀	1	6.3	19.8	24.0-17.4	10.2	12.0- 9.4
♂	5	9.0	22.1	25.0-20.0	10.7	14.0- 9.0
♀	5	11.0	21.3	25.0-18.3	10.5	12.3- 9.0
♀	11	14.0	26.4	30.0-23.0	13.1	15.0-11.0
♂	11	15.0	24.9	30.4-22.0	13.1	13.7-11.6
♂	16	18.9	25.3	29.0-22.0	13.1	14.0-12.0
♀	16	19.0	23.1	25.0-20.3	11.2	14.0-10.0
♀	20	295.0	23.6	27.0-19.4	11.8	16.0-10.4
♀	20	317.0	26.4	29.0-24.0	12.5	14.0-11.0
♂	25	238.0	26.6	31.0-23.0	12.6	14.0-11.0
♀	25	25.5	27.3	31.0-24.7	12.7	14.0-11.0
♀	29	16.4	24.8	26.0-22.7	12.2	14.0- 9.4
♂	29	40.7	27.1	30.0-24.7	12.0	14.0-10.0
♀	42	43.5	27.2	31.0-24.7	13.2	16.0-11.0
♂	42	61.4	27.0	30.0-24.7	13.4	16.0-12.0
♀	48	49.7	27.0	29.0-24.7	13.1	15.0- 9.0
♂	48	105.1	29.0	33.0-26.4	13.4	15.0-12.6
♂	60	51.6	27.2	30.0-24.0	13.2	15.0-12.4
♀	62	53.8	27.1	30.0-24.0	13.1	15.0-11.6
♀	80	83.7	26.6	29.0-25.7	12.8	13.7-11.6
♂	81	63.3	27.4	31.5-25.7	13.3	15.6-11.0
♀	88	73.0	29.2	31.0-26.0	13.2	14.6-11.0
♂	89	143.5	32.4	36.7-28.5	13.0	14.6-11.0
♀	124	107.0	30.5	33.0-29.0	13.8	16.0-13.0
♂	124	151.1	27.1	28.5-25.0	13.0	14.0-11.7
♀	171	123.8	30.9	33.7-29.0	12.8	13.7- 9.7
♂	171	198.2	27.0	32.4-24.7	13.1	14.0-12.4
♀	250	98.0	30.6	33.0-26.6	14.2	16.0-14.0
♂	250	230.0	36.8	39.0-35.6	15.4	18.0-14.4
♀	365	170.6	31.4	38.0-28.5	13.5	15.6-12.3
♂	365	186.0	29.6	33.7-26.6	13.5	16.0-12.0
♀	540	151.3	30.7	34.4-28.3	13.4	14.7-11.0
♀	570	127.1	33.4	36.7-29.0	14.3	16.0-14.0

At birth or during the first days of life there are found among the young cells a few advanced cells which appear conspicuously different from the rest. In these advanced cells the cytoplasm may be already differentiated, even at birth. The stainable Nissl granules, which are of course much finer than those found at later ages, are evenly but distinctly distributed through the entire contents of the cell. Among these granules some clear spaces appear which seem to indicate the differentiation of the homogeneous cytoplasmic mass, and this change in the advanced cells must have commenced during fetal life.

When the young cells begin to develop, there is the same differentiation of the cytoplasmic mass, and the stainable bodies arrange themselves in the same way as those seen in the advanced cells. Hereafter more differentiation will be found in them and they grow to resemble the advanced cells in appearance.

Taking this as the starting-point in the morphological development, we see among the comparatively large cells in the ganglion four types which probably appear one after the other as here given in the course of growth.

Type 1. The advanced cells and the cells which are transforming into advanced cells, as described above, belong to this type. There is a beginning of aggregation of the Nissl granules and a growth of the unstainable ground-substance in the cells. This type is common during the first twenty days of postnatal life (fig. 2).

Type 2. The Nissl bodies are larger than in type 1 and aggregated at the periphery of the cells, forming a ring within which is a comparatively clear portion of the ground-substance surrounding the nucleus. The Nissl bodies stain much darker than in type 1. The nuclear membrane, the nucleoli, and the reticular structure in the nucleus are distinctly visible. There are frequently two or more nucleoli in one nucleus. This type is common in the period between twenty and sixty days (fig. 3), but may also be found at birth (fig. 1).

Type 3. Instead of being distributed at the periphery, the Nissl bodies are aggregated around the nucleus, leaving a rather clear space at the periphery of the cell. In some of the cells they

are more crowded at certain regions close to the nucleus, forming dark masses, but some of them may be loosely scattered toward the periphery. It is in this type of cell that difficulties have often been encountered in making out the boundary between the cell wall and the supporting tissue, because the unstained ground-substance is chiefly distributed at the periphery of the cell. This type is common after twenty days of age, but is not infrequently found after sixty days (fig. 4).

Type 4. The cells resemble the first type in the arrangement of Nissl bodies, but the stainable bodies are much coarser. There is a considerable evenness in their distribution, though here and there we find a larger dark stainable mass resulting from their aggregation. Whether this type is developed from the preceding type through modifications in the course of development or whether it is directly derived from type 1, without undergoing the various changes as in types 2 and 3, is a matter to be settled through more detailed investigation (fig. 5). This type is characterized by the dense appearance of Nissl bodies throughout the entire cell body, not leaving much space for the ground-substance, and is common at the age of 124 days and later.

In interpreting these several types it is to be recalled that the cells of this ganglion have several different functions and there always remains the possibility of a correlation between function and morphology.

Besides the four types of cells described above, binuclear cells are found at all ages until the rat is very old. In recording them, special care needs to be taken. As the cell wall of the sympathetic cell is at times difficult to distinguish, two uninuclear cells in close contact with each other may frequently resemble one cell with two nuclei. In order to avoid error due to such misleading appearances, the precaution has been taken to use an oil-immersion lens in distinguishing the true binuclear cells from those which resemble them. The cells which have their cytoplasm discontinuous somewhere between the nuclei or a constriction at the middle, either slight or pronounced, as the one figured by Apolant ('96, Majer's 'cell bridge,' fig. 8, pl. XXIII), were not considered as of the true binuclear type.

TABLE 5

Giving the number of the cells with two nuclei and of the cells showing pigment, at different ages. Superior cervical sympathetic ganglion—albino rat.

In each case the numbers are for one ganglion only

SEX	AGE	BODY WEIGHT	BODY LENGTH	NUMBER OF BI-NUCLEAR CELLS	CELLS WITH PIGMENT
	<i>days</i>	<i>grams</i>	<i>mm.</i>		
♂	1	5.6	50	2	0
♀	1	6.3	51	2	0
♂	5	9.0	63	1	0
♀	5	11.0	65	2	0
♂	11	15.0	77	1	0
♀	11	14.0	73	2	0
♂	16	18.9	83	1	0
♀	16	19.0	81	1	0
♂	20	31.7	102	2	0
♀	20	29.5	99	4	0
♂	25	23.8	93	4	0
♀	25	25.5	95	3	0
♂	29	40.7	112	3	0
♀	29	16.4	82	3	0
♂	42	61.4	129	5	0
♀	42	43.5	105	12	0
♂	48	105.1	156	4	0
♀	48	49.7	120	2	0
♂	60	51.6	124	5	0
♀	62	53.8	117	5	0
♂	81	63.3	128	3	0
♀	80	83.7	142	12	0
♂	89	143.5	173	7	0
♀	88	73.0	135	1	1
♂	124	151.1	174	15	8
♀	124	107.1	157	6	0
♂	171	198.2	192	2	4
♀	171	123.8	159	9	0
♂	250	230.0	207	4	0
♀	250	98.0	160	5	3
♂	365	186.0	203	6	3
♀	365	170.6	186	4	2
♂	540	151.3	184	2	4
♀	570	127.1	169	3	13

Average of binuclear cells: $\begin{cases} \sigma^{\circ} 4.0 \\ \varphi 4.4 \end{cases}$

Every one of the cells recorded in table 5 had an unbroken layer of Nissl granules around the two nuclei, and at the middle of the cell there existed absolutely no trace of any partition whatsoever which might suggest the contiguous surfaces of two cells closely grown together. Figures 6 and 7 show the binuclear cells in a very young and in a comparatively old rat, respectively.

If we determine, by direct measurement, the nucleus-plasma relation in this particular older cell (fig. 7), contrasting the volume of both nuclei with that of the cytoplasm, we find a ratio of 1 : 5.0. This is almost as low as the ratio at birth, and indicates that we are dealing with an increase in the nuclear mass not accompanied by a corresponding increase in the cytoplasm. This, so far as it goes, is an argument against the suggestion that we have here two cells that are fused.

According to table 5, the occurrence of binuclear cells is not related to sex. In many cases the numbers of these cells in both sexes are equal or almost equal. There appears, however, to be an increase in their number toward middle age, ranging from sixty days to 365 days, with a possible decrease later.

Apolant found cells of the binuclear type in the superior cervical ganglion of an embryo rabbit three weeks old, and states that such cells persist in the older animal, when the cells have been completed anatomically and physiologically. According to him, this is the result of direct nuclear division; about half of the binuclear cells being formed during embryonic life and the remainder later. It is not the purpose of this paper to deal with the function and origin of this type of cells. Their appearance in the postnatal stages of the rat, as recorded in table 5, agrees with what Apolant points out as the course of the development of the cells in the later ages of the animal. Carpenter and Conel ('14) noted this type of cells in considerable number in the rabbit, guinea-pig, muskrat, and porcupine, but rarely, if ever, did they find them in the sympathetic ganglion of the rat.

As these authors' observations were made most probably on one or on only a few stages of the rat, the small number of such cells in the entire ganglion justifies their statement, in a way but nevertheless the presence of the binuclear cells in the superior cervical sympathetic of the rat is beyond question.

Incidentally, pigmented cells have been noted in the superior sympathetic ganglion of the albino rat. The cells of comparatively young animals, from birth to eighty days, are entirely free from pigment. At the beginning of puberty we occasionally find pigment in one or two cells in an entire ganglion. The number of the pigmented cells tends to increase as age advances, as recorded in table 5. Some of the cells are only partly pigmented; a few are completely covered with these granules, the nucleus remaining unaffected, while others are totally pigmented, including the nucleus. The pigments appear yellow brown, or, black in color, but whether this is merely a result of their relative abundance or whether there are several sorts of pigment has not been determined. The whole question of pigment in the Albino nervous system seems worthy of a special investigation.

INCREASE IN THE NUMBER OF THE LARGE CELLS

The increase in the number of the large cells in the ganglion during the first twenty days is an important event. This is chiefly due to the rapid increase in diameter of the young cells after ten or fifteen days of age. The large cells measure 19 to 25 μ in diameter, and are loosely scattered and intermingled with small cells, as seen in each section. Disregarding their finer differences, such a group of cells consists of three kinds:

1. The advanced cells. During embryonic development it is known that the sympathetic trunks are formed through the migration of some cells which pass from the spinal cord along the paths of the communicating rami (Kuntz, '10). The advanced cells in the superior cervical ganglion are the forerunners of the neurones which come to this locality in "skirmish order—much in advance of the others" and "they represent but a fraction of the final number of large cells" (Donaldson, '17). The number of these cells during the first twenty days varies from one to eight in the entire ganglion.

2. The moderately large cells. These cells constitute an intermediate group between the advanced cells and the small cells in the same ganglion during the first ten days of age. They are not different from other younger cells in general structure and

TABLE 6

Increase in number of large and advanced cells, 19 to 25 μ in diameter, during the first twenty days of life. The ratios in the increase in the total number for both sexes between one day and twenty days stand at the foot of the column. Superior cervical sympathetic ganglion—albino rat

SEX	AGE	BODY WEIGHT	NUMBER OF LARGE CELLS AND OF ADVANCED CELLS
	days	grams	
♂	1	5.6	188
♀	1	6.3	174
♂	5	9.0	289
♀	5	11.0	306
♂	11	15.0	291
♀	11	14.0	301
♂	16	18.9	760
♀	16	19.0	584
♂	20	31.7	2508
♀	20	29.5	2248

Ratios: $\left\{ \begin{array}{l} \text{♂ } 13.34 \\ \text{♀ } 12.91 \end{array} \right.$

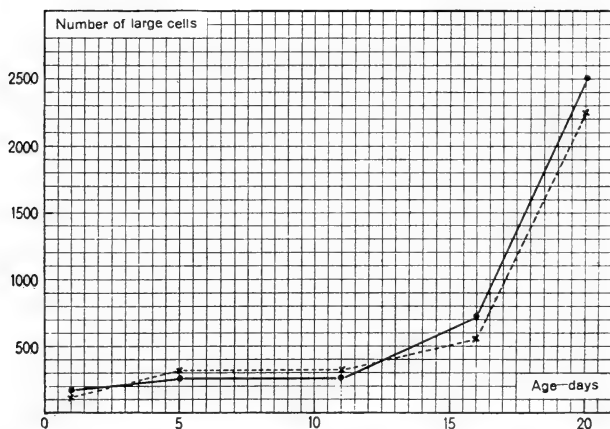


Chart 3 Based on table 6 and showing the number of large cells present in the superior cervical sympathetic ganglion of the albino rat from birth to twenty days of age. Males ———. Females -----.

form, but they are distinguishable, owing to their larger size. It is this group of cells which will appear later as the advanced cells.

3. The growing small cells. These cells are small during the first five days after birth, but some of them grow very fast toward the end of twenty days, to a size equal to that of the other large cells. There is a constant increase in the number of these smaller cells which are growing.

For the determination of the rate of increase in the number of the large cells, 19 to 25 μ in diameter, counting was undertaken. The cells counted comprised those just described under 1 and 2. Since the same large cell does not appear in two successive sections, repetition in counting them is easily avoided. Table 6 gives the numbers of these cells. Based upon these numbers, the graphs in chart 3 were plotted on age. In chart 3 the male has a slightly higher rate of increase than has the female after twelve days. When the animal reaches sixteen days, both sexes show a more rapid increase, and the difference between them becomes more evident. If the data are plotted in a like manner on the body weight, they show similar relations. On the whole, then, the data show that the increase of large cells during the first sixteen days is relatively slow and afterward increasingly rapid. Between the age limits here given the increase in the number of large cells—sexes combined—is about thirteen-fold.

THE TRANSFORMATION OF THE YOUNG CELLS

During the later period of development there remain in this ganglion a number of young cells which, in contrast with the large cells, are slow in growth and which retain their neuroblastic appearance for a considerable length of time (fig. 1). As already noted, some nerve cells are precocious and many of them have attained their maximum size at the end of twenty to twenty-five days. It is most probable that the young cells found after twenty-five days of age are largely rudimentary elements, and some of them will never grow to the same size as the others. Yet some development is going on in both their structure and size, as is indicated by the constant decrease of their number

TABLE 7

Showing the changes in the number of young cells from the period just prior to puberty to the end of one year. Superior cervical sympathetic ganglion—albino rat

SEX	AGE	BODY WEIGHT	NUMBER OF YOUNG CELLS
	<i>days</i>	<i>grams</i>	
♂	60	51.6	470
♀	62	53.8	471
♂	89	143.5	362
♀	88	73.0	345
♂	124	151.1	326
♀	124	107.1	252
♂	171	198.2	242
♀	171	123.8	207
♂	365	186.0	105
♀	365	170.6	102

$$\text{Ratios: } \begin{cases} \text{♂} & 1:0.224 \\ \text{♀} & 1:0.217 \end{cases}$$

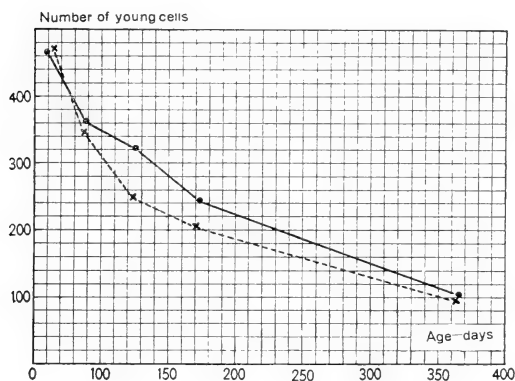


Chart 4 Based on table 7. Showing the changes in the number of young cells in the superior cervical sympathetic ganglion of the albino rat between sixty and 365 days of age. Males ———, Females -----.

toward the end of one year. A study of the rate in the decrease of the young cells will serve as a means of measuring this change at later ages. For this purpose counting was undertaken. Because of their considerable number during the early prepubertal stage, as well as their small size and irregular distribution, it is almost impossible to obtain a satisfactory value by a single count, so that a second and a third count were usually made.

The numbers recorded in table 7 represent averages of three counts of young cells in each ganglion. The cells, selected and counted as young cells, have the following characters: They are 5 to 10 μ in long diameter; more or less pyriform, and the cytoplasm is little differentiated.

The graphs in chart 4 represent the numbers of the cells as given in table 7, on age. The decrease in number at first shows no tendency for one sex to outrun the other, but a difference appears soon after puberty, and such a difference in decrease of the young cells between the male and the female persists till the end of one year. The young cells of the female rat in relation to age are transformed more rapidly than those of the male; that is, these cells grow faster in the female. This phenomenon is in accord with what has been seen in the growth of large cells in diameter, as shown in charts 1 and 2.

DISCUSSION

There is reason to think that at birth the full number of cells in the superior cervical sympathetic of the albino rat has been attained and that no more cells wander in and mitosis is finished. These cells appear to persist throughout the span of life.

Postnatal development of these cells consists in the enlargement of all parts of the neuron accompanied by differentiation. In the nucleus there is less change in size than in the cell body. The increase in number of nucleoli has been frequently noted, but it is not within the scope of the present paper to discuss this point.

Bringing the observations together, we see that the male and the female do not differ clearly from each other in the growth of these nerve cells until the animal has become sexually mature.

The rate of increase in the number of the large cells in the superior cervical ganglion is, if anything, a little lower in the females before twenty days and a little higher later. On the other hand, the increase in size shows only chance variations during the first seventy days. These variations are subject to the influence of both age and body weight of the young animal, but puberty is attained, sex begins to be significant in addition to the other two factors.

If there is not too much difference between the ages and the body weights of the male and of the female, then the difference found in the quantitative development of the cytoplasm between the two may be attributed to this influence of sex. In table 8

TABLE 8

Giving, according to sex, the average computed diameters of the cell and the nucleus for three groups of body weights of albino rats. In the last column are given the ratios between the cell and the nucleus diameters. Data condensed from table 2

SEX	NUMBER OF CASES	BODY WEIGHT	DIAMETERS		RATIO OF DIAMETER OF NUCLEUS TO CELL
			Cell	Nucleus	
		<i>grams</i>	μ	μ	
♂	4	5.6-18.9	22.9	12.1	1:1.89
♀	5	6.3-19.0	23.1	11.4	1:2.03
♂	7	23.8-105.1	27.2	12.9	1:2.11
♀	9	25.5-107.0	27.7	13.1	1:2.11
♂	5	143.5-230.0	30.6	13.6	1:2.25
♀	4	123.8-170.6	31.6	13.5	1:2.34

is given a condensed statement of the cell measurements according to sex, based on body weights as these appear in table 2. For the cell body the values are in favor of the female for all three groups.

After puberty the sympathetic neurons in the female tend to have larger cell bodies and the small cells transform more rapidly.

At the moment it would not be wise to infer that similar relations would be found in other sympathetic ganglia or in other strains of rats; nevertheless, as they stand, the results agree with the suggestion of Dunn ('12) that the mass of the peripheral nervous system in the female albino rat is greater in proportion to the body weight than in the male.

In table 9 are given the amounts by which the cell diameters of the females differ from those for the males at four ages after eighty days. The mean excess for the females is about 6.9 per cent, which represents approximately an excess of 20 per cent in volume. When a corresponding comparison is made for the diameters of the nuclei in these four groups, the average difference according to sex is found to be zero.

TABLE 9

Showing, in four age groups, the absolute and percentage difference in the cell diameters of the largest cells in the superior cervical sympathetic ganglion of the female albino rat as compared with the male, based on the values in table 1. Because of the great difference in the body weights, the data for the group at 250 days are omitted

AGE	CELL DIAMETER IN THE FEMALE DIFFERS FROM THAT IN THE MALE BY	
	Absolute μ	Percentage
<i>days</i>		
80	-0.8	- 3.0
124	+2.6	+ 9.6
171	+3.9	+14.4
365	+1.8	+ 6.0
Average		+ 6.9

On the size of these cells in the inbred albino rat

To determine whether the size relations according to sex which have just been described for albino rats belonging to the so-called 'standard strain' are generally found, a series of inbred Albinos was examined, for comparison.

The specimens used in this study were furnished by Dr. Helen D. King. These rats had been closely inbred for thirty-four to thirty-five generations. Seven pairs were used, ranging from eighty-nine days to 154 days and each pair was from the same litter. The preparation of the specimens was made in the same manner as that for the series just described.

The records on sex, age, body weight and length and the measurements of the cells and nuclei are given in the following table 10.

Using table 10, chart 5 was plotted on age. The graphs show a slight difference in the size of the cells of the male and female. The male, as indicated by the graphs, seems to have a better growth in the cytoplasm than the female of the same age, but the difference is small and cannot be considered as primarily

TABLE 10

Data on the inbred albino rats from the colony of Doctor King. Diameters of largest cells in the superior cervical sympathetic ganglion—on age

SEX	AGE	BODY WEIGHT	BODY LENGTH	DIAMETERS		RATIO OF DIAMETER OF NUCLEUS TO CELL
				Cell	Nucleus	
				μ	μ	
♂	89	144	176	21.90	12.90	1:1.69
♀	89	100	156	21.60	13.00	1:1.66
♂	103	232	202	24.60	14.05	1:1.75
♀	103	203	189	24.20	13.25	1:1.82
♂	123	206	193	24.70	13.12	1:1.88
♀	123	176	187	24.21	13.14	1:1.84
♂	116	140	177	23.21	12.60	1:1.84
♀	116	110	172	22.81	12.50	1:1.82
♂	131	310	220	28.80	14.00	1:2.07
♀	131	205	192	26.00	13.50	1:1.92
♂	136	179	188	24.80	12.79	1:1.94
♀	136	148	182	25.80	13.79	1:1.87
♂	154	251	212	27.21	15.21	1:1.79
♀	154	188	189	26.00	13.45	1:1.93

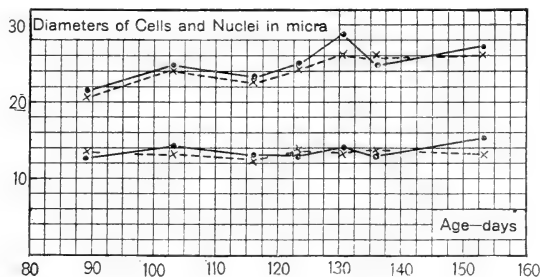


Chart 5 Based on table 10. Showing the computed diameters of the cells and their nuclei, according to sex, on age in days (inbred Albino). Male——— Female-----

due to sex, because in each pair the male has a greater body weight than the female.

In table 11 the data have been arranged according to body weight. Using these, chart 6 was plotted. In only one instance is the value for the female below that for the male in the case of the cells, while the female values for the nuclei are always above those for the male.

TABLE 11

Giving the computed diameters of the cells and their nuclei arranged according to body weight. The same data as in table 10. Inbred albino rats.

SEX	AGE	BODY WEIGHT	BODY LENGTH	DIAMETERS		NUCLEUS PLASMA RATIOS
				Cell	Nucleus	
				μ	μ	
♀	89	100	156	21.60	13.00	1:2.6
♀	116	110	172	22.81	12.50	1:5.1
♂	116	140	177	23.21	12.60	1:5.2
♂	89	144	176	21.90	12.90	1:3.9
♀	136	148	182	25.80	13.79	1:5.5
♀	123	176	087	24.21	13.14	1:5.2
♂	136	179	188	24.80	12.79	1:6.3
♀	154	188	189	26.00	13.45	1:6.2
♀	103	203	189	24.20	13.25	1:5.1
♀	131	205	192	26.00	13.50	1:6.1
♂	123	206	193	24.70	13.12	1:5.2
♂	103	232	202	24.60	14.05	1:4.0
♂	154	351	212	27.21	15.21	1:4.7
♂	131	310	220	28.80	14.00	1:7.7

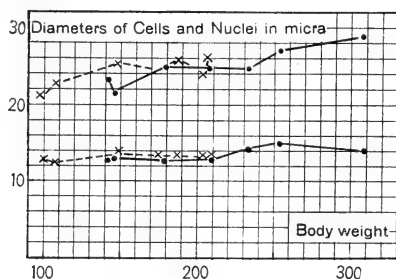


Chart 6 Based on table 11 and giving the computed diameters of the cells and their nuclei, according to sex, on body weight (inbred Albinos).
Male —————. Female - - - - -.

As shown in table 10, the female is smaller in size than the male at each age, the female at equal body weight must therefore be older, consequently the cells might have a slightly larger diameter as the result of age, but the difference is small. It would be fair to say, therefore, that the cells, as well as the nuclei, as shown in chart 6, do indicate a sex difference although it is slight.

This result supports in principle the earlier findings on the standards rats.

TABLE 12

To illustrate the way in which the 'inbred' differ from the 'standard' albino rats in respect of the diameters of the largest cervical sympathetic cells and their nuclei. Data from tables 1 and 11

AGE	BODY WEIGHT	DIAMETERS		
		Cells	Nucleus	
		μ	μ	
89	122*	21.75	12.95	Inbred albino rat
124	129	28.80	13.40	Stock albino rat
Difference {		-7.05	-0.45	
		-32.0	-4.0	
123	191	24.45	13.13	Inbred albino rat
171	161	28.90	13.00	Stock albino rat
Difference {		-4.45	+0.13	
		-19.0	+1.0	

*The values given are the average for the male and female in each instance.

In table 12 is given a comparison of the diameters of the cells and nuclei of the inbred Albino with those of the stock albino rat. The data for the latter have been selected from tables 2 and 11. The sexes are combined.

According to table 12, the inbred has its largest cells in the superior cervical sympathetic ganglion decidedly smaller than those in the stock albino rat of approximately the same body weight. The nucleus, however, shows only a little difference between the two forms, though this difference is in the same direction.

In her studies on inbreeding, King ('18) states that the closest form of inbreeding, continued for many generations, has not caused a diminution in the average body weight of the inbred rat at any age, and that through the selection of the largest and most vigorous animals for mating, inbred rats are superior in body size to the stock animals reared under similar environmental conditions. Nevertheless, our data as they stand indicate that in the inbred rats the largest cells in this ganglion are clearly smaller in size than in the standard strain. It seems best not to comment on this relation until studies have been made on the wild Norway, and these I hope soon to undertake.

SUMMARY

A. Based on the data for the 'standard' strain

1. Between birth and maturity the largest cells in the superior cervical sympathetic ganglion increase about 55 per cent in diameter, while the increase in the nuclei is less than half of this amount.

2. The growth occurs in two phases: the first phase of rapid growth ends at about twenty-five days and the second phase of less rapid growth continues to the end of the record. The present data do not show a marked alteration in rate at puberty.

3. The size of these cells is more closely related to the body weight than to the age of the rat, but there is a marked tendency after puberty for the females to have slightly larger cells than the males of the same age.

4. The nucleus-plasma ratio increases from 1 to 4 at birth to about 1 to 12 at maturity.

5. At maturity the large cells may be classified in three groups: 1) those with Nissl bodies accumulated at the periphery of the cell; 2) those with large masses of Nissl bodies accumulated around the nucleus; 3) those with larger Nissl bodies mingled with small ones, and more or less evenly distributed within the cell. Moreover, a few binuclear cells are found, and in the older rats some pigmented cells are present.

6. Taking the ganglion as a whole, the large or advanced cells may be present, though in very small numbers, even at birth. This number is slowly increased up to about the fifteenth day, after which the increase is more rapid. Correlated with this is a decrease in the number of small cells which are transformed into the large cells. This transformation continues during the first year and probably throughout life. It appears to occur slightly earlier in the female.

B. Based on the data for the 'inbred' strain

7. The inbred rats ranged from 89 to 154 days in age. When the values for the diameters of the cells and of the nuclei were plotted on age, these values were greater for the males. The males were also consistently greater in body weight. When the values were plotted on body weight, the values for the females were in general above those for the males. In this case the females were older than the males with which they were compared. It seems probable that at like ages and like body weights, the females would show slightly higher values, but this may be merely an expression of precocity in this growth change in the females.

8. When these cells in the inbred rats are compared with those in the standard animals, table 12, it is seen that while the nuclei differ but little in diameter, the cells in the standard Albinos have a diameter some 25 per cent greater than that found for the inbred cells. It is to be noted that this difference in diameter would make the volume of these cells in the standard Albino about twice that in the inbred, while the nuclei differ but slightly. This difference in the cells is definite, but the significance of it is not discussed here.

9. The ratios of the diameter of the nucleus to that of the cell are in the inbred distinctly less than in the standard Albino, within the same age limits. Compare data in table 1 with those in table 10.

10. The nucleus plasma ratios in the inbred are only about half as great as in the corresponding cells of the standard Albino. Compare data in table 2 with those in table 11.

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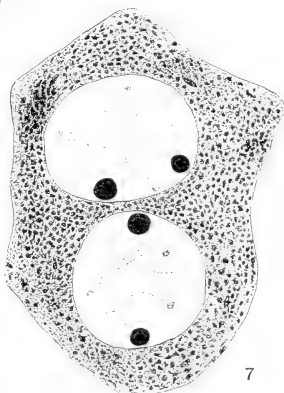
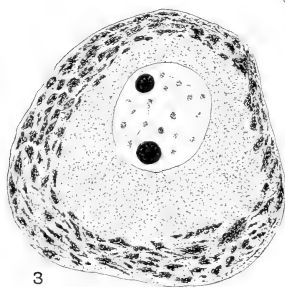
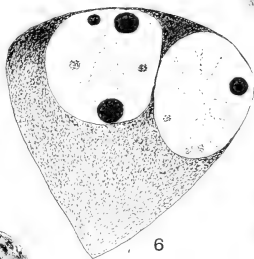
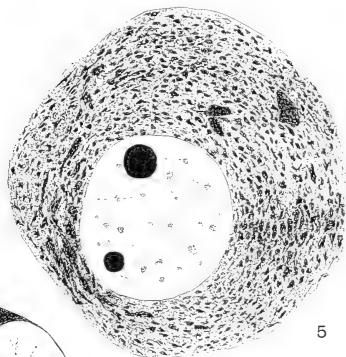
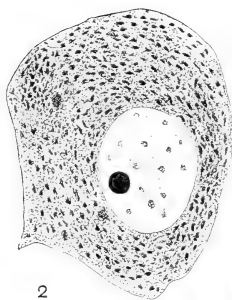
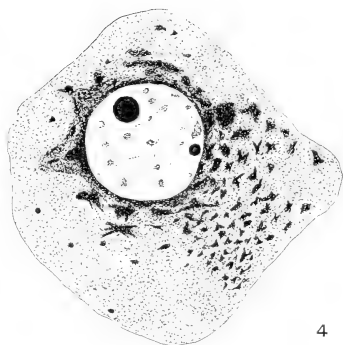
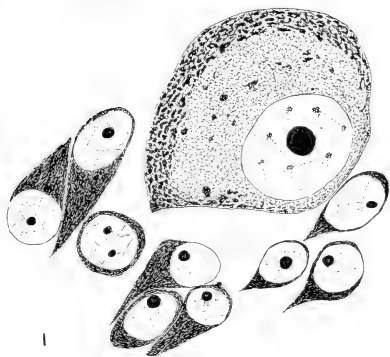
PLATE

PLATE 1

EXPLANATION OF FIGURES

Cells from the superior cervical sympathetic ganglion of the albino rat. Figures 1 to 7 magnified in plate by 2000.

- 1 An advanced cell and several young cells; male, one day old.
- 2 An advanced cell with Nissl bodies evenly distributed; male, five days old.
- 3 A cell with Nissl bodies accumulated at the periphery, common between twenty days and sixty days; female, twenty days.
- 4 A cell with Nissl bodies accumulated around the nucleus, common between twenty days and sixty days and also found at later ages; male, sixty days old.
- 5 A cell with larger Nissl bodies more or less evenly distributed common after one hundred days; female, 124 days old.
- 6 A binuclear cell; female, one day old.
- 7 A binuclear cell; male, one year old.





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Resumen por el autor, Chi Ping.

Sobre el crecimiento de las mayores células nerviosas del ganglio cervical superior del simpático en la rata noruega.

Las mayores células de este ganglio crecen de un modo muy semejante al observado en el caso de la rata albina. Estas células en la rata noruega presentan núcleos con los mismos diámetros que las de la rata albina, pero los cuerpos celulares son algo más pequeños. La relación núcleo-plasmática es menor que en la rata albina. Las células binucleares son menos numerosas en la rata noruega, pero las células pigmentadas son solamente un poco más numerosas. Existen unas cuantas células vacuoladas que parecen ser características de las ratas noruegas salvajes más viejas. En las albinas "inbred," comparadas con el tronco tipo, estas células poseen núcleos del mismo tamaño, pero el diámetro celular es mucho menor; por esta causa sus relaciones núcleo-plasmáticas son muy bajas. No puede decirse todavía si estas diferencias se deben a la domesticación o es simplemente una particularidad de este tronco.

Translation by José F. Nonidez
Cornell Medical College, New York

ON THE GROWTH OF THE LARGEST NERVE CELLS IN THE SUPERIOR CERVICAL SYMPATHETIC GANGLION OF THE NORWAY RAT

CHI PING

The Wistar Institute of Anatomy and Biology

FIVE CHARTS

INTRODUCTION

This study is a continuation of my first work "On the growth of the largest nerve cells in the superior cervical sympathetic ganglion of the albino rat from birth to maturity" (Ping, '21).

In that paper the significance of the age, the size of the animal, and of sex on the growth of the cells was examined, and an unexpected difference in the size of these cells was found in the 'inbred' as contrasted with 'standard' strain of Albinos. It was deemed important, therefore, to examine the Norway rat in the same way in order to determine how the size and the growth changes in these cells were related in the wild Norway to those found in the two domesticated albino strains.

MATERIAL AND TECHNIQUE

The eighty-five specimens of the wild Norway used in this study belong to two groups from different sources. One group, comprising twenty-two individuals, was reared at The Wistar Institute, and the ages of these animals range from one day to 134 days.

The other group of sixty-three animals was collected from different localities in Philadelphia and its vicinity, and the ages of these are unknown. Their body weights range from 37 to 402 grams, corresponding to ages from twenty days to three years, as generally estimated.

The technique employed in preparing the specimens is the same as that given in my former paper. The trapped rats were carefully examined when dissected, and all the specimens used were considered normal. As the ages of the trapped rats were unknown, the determination of the percentage of water in the brain was made in each case, since by this means the approximate age of the animal may be estimated, as pointed out by Donaldson ('10), Donaldson and Hatai ('11, and '16).

TABLE 1

Giving according to body weight the computed diameters of the largest cells and nuclei in the superior cervical ganglion of the Norway rat. Sexes separated. Data condensed. Thirteen groups

FORTY-THREE MALES				FORTY-TWO FEMALES			
Number of cases	Body weight average	Computed diameter		Computed diameter		Body weight average	Number of cases
		Nucleus	Cell	Cell	Nucleus		
	grams	μ	μ	μ	μ	grams	
1	6	9.9	17.2	16.5	9.6	6	1
3	15	12.9	22.8	22.5	12.5	14	3
5	35	12.4	23.4	23.2	11.7	28	2
3	76	13.0	25.5	25.2	13.0	31	1
3	104	13.1	26.5	24.3	12.7	51	8
2	117	13.2	27.1	26.0	13.0	73	2
3	157	13.3	26.1	24.9	13.0	103	4
3	186	13.4	27.9	28.3	13.3	157	4
5	220	13.6	29.7	27.7	13.0	179	6
4	244	14.0	31.3	26.6	12.8	192	3
3	276	13.5	33.3	27.8	12.7	214	3
5	323	14.0	33.0	31.6	13.7	227	2
3	385	13.7	31.0	32.1	13.9	258	3

In measuring the cells and the nuclei of the superior cervical ganglion I followed the same procedure as in my former study of the Albino. The data represented by the computed diameters of the cells and nuclei, and their ratios, together with the sex, age, body length, and body weight, as well as the percentage of water in the brain, were tabulated in the first instance for each individual according to increasing body weight, but these data have been condensed for the purpose of this paper—and only the condensed tables will be used for discussion. The

individual data are on file in the archives of The Wistar Institute and available there for reference.

The values for the diameters of the cells and their nuclei are the averages of measurements on the twenty largest cells in each ganglion.

GROWTH OF THE CELLS

A. *In relation to body weight*

The computed diameters of the cells and the nuclei of the eighty-five cases have been condensed to thirteen groups for

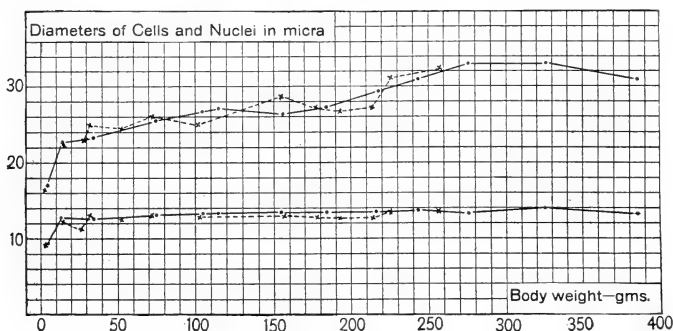


Chart 1 Based on table 1 and giving the computed diameters of the cells and their nuclei according to sex—on body weight in grams. Wild Norway rat. Males ——— Females - - - - -

each sex and arranged according to body weight as shown in table 1. From these data chart 1 was plotted.

The graphs in chart 1 show both the cell bodies and the nuclei as growing rapidly up to a body weight of 15 grams, but after that period they grow more gradually. The graphs for the males and females run close together at all body weights, and there is no indication of the difference according to sex shown in chart 2 for the Albino (Ping, '21).

B. In relation to body length

Following the procedure just used, the condensed data have been arranged to show the relations of the cell diameters according to increasing body length, and are given in table 2. There are thirteen groups for the males and eleven for the females. The corresponding graphs are plotted in chart 2. According

TABLE 2

Giving according to body length the computed diameters of the largest cells and nuclei in the superior cervical ganglion of the Norway rat. Sexes separated. Data condensed

MALE				FEMALE			
Number of cases	Body length average	Computed diameter		Computed diameter		Body length average	Number of cases.
		Nucleus	Cell	Cell	Nucleus		
	mm.	μ	μ	μ	μ	mm.	
1	53	9.9	17.2	16.5	9.6	50	1
2	71	12.5	21.9	22.5	12.5	71	3
1	84	13.8	24.4	23.9	12.1	103	3
2	104	12.6	23.6	23.4	12.8	114	1
3	112	12.3	23.3	24.6	12.8	123	5
3	144	12.9	25.5	24.7	12.7	130	3
5	167	13.1	26.8	25.7	12.9	146	3
5	189	13.3	26.4	27.3	13.2	184	12
6	209	13.5	29.8	28.3	12.8	202	6
3	214	14.8	32.6	28.9	13.5	213	2
5	225	13.5	32.6	31.5	13.7	222	3
4	233	13.8	32.4				
3	251	13.7	31.1				

to the graphs, there seem to be two periods in which the diameter of the cell is showing a rapid growth; one period at a body length of about 80 mm. and the other at a body length of about 200 mm. Without entering into the details, it will be sufficient to point out that both periods are those in which the body weight is increasing rapidly in relation to the body length, and it is probably the influence of the body weight which appears in the graphs. At the same time there is no sex difference to be seen in the diameters of either the cells or their nuclei.

C. In relation to observed age

The rats with known ages form a separate series for the present discussion. The computed diameters of the cells and the nuclei are given in table 3 according to age. Examination of the table reveals that the cells and nuclei grow comparatively fast during the first twenty-five days of life. In order to show the contrast between the early growth and that which follows, data selected from table 3 have been arranged in table 4.

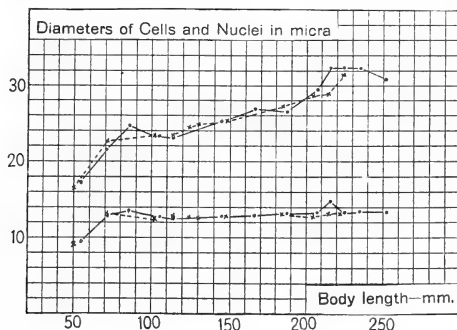


Chart 2 Based on table 2 and giving the computed diameters of the cells and their nuclei according to sex—on body length in millimeters. Wild Norway rat. Males — Females - - - - -

Table 4 shows that at the end of twenty-five days the cells and the nuclei have increased in diameter 1.31 and 1.14 times, respectively, for the male, and 1.43 and 1.25 times for the female; while the increase from 25 days to 134 days—a period which is more than five times as long—is 1.19 for the cells and 1.21 for the nuclei of the male and 1.04 for the cells and 1.06 for the nuclei of the female.

The graphs in chart 3 illustrate the fact that during the first twenty-five days there occurs a sudden and rather irregular increase, which is followed by a slow advance. No clear indication of a difference according to sex is to be seen.

TABLE 3

Giving according to age the computed diameters of the cells and nuclei in the superior cervical ganglion of the Norway rat. Sexes separated. From detailed record

SEX	AGE	COMPUTED DIAMETERS		RATIOS OF DIAMETER NUCLEUS TO DIAMETER OF CELL
		Cell	Nucleus	
	<i>days</i>	μ	μ	
♂	1	17.19	9.85	1 : 1.74
♀	1	16.51	9.60	1 : 1.72
♂	5	22.20	12.41	1 : 1.79
♀	5	22.40	12.30	1 : 1.82
♂	10	21.66	12.50	1 : 1.74
♀	10	22.21	12.60	1 : 1.75
♂	15	24.40	13.82	1 : 1.76
♀	15	22.82	12.60	1 : 1.81
♂	19	26.00	13.86	1 : 1.88
♀	19	25.21	13.00	1 : 1.94
♀	25	22.61	11.25	1 : 2.01
♀	25	23.80	12.06	1 : 1.86
♂	28	26.00	13.40	1 : 1.94
♂	31	21.21	11.25	1 : 1.89
♂	31	21.02	10.55	1 : 1.99
♀	60	27.22	14.20	1 : 1.93
♂	65	25.00	13.04	1 : 1.92
♀	65	26.24	13.49	1 : 1.95
♂	80	23.25	13.40	1 : 1.73
♀	80	23.41	12.80	1 : 1.82
♂	134	27.00	13.70	1 : 1.97
♀	134	24.70	12.85	1 : 1.84

TABLE 4
Increase in diameters of cells and nuclei at three different ages

SEX	AGE	DIAMETERS	
		Cell	Nucleus
	<i>days</i>		
♂	1	17.19	9.85
♀	1	16.61	9.60
♂	25	22.61	11.25
♀	25	23.80	12.06
♂	134	27.00	13.70
♀	134	24.70	12.85
Ratios between 1 day and 25 days		$\left\{ \begin{array}{l} \text{♂} \\ \text{♀} \end{array} \right.$	$\left\{ \begin{array}{l} 1 : 1.14 \\ 1 : 1.25 \end{array} \right.$
Ratios between 25 and 134 days		$\left\{ \begin{array}{l} \text{♂} \\ \text{♀} \end{array} \right.$	$\left\{ \begin{array}{l} 1 : 1.21 \\ 1 : 1.06 \end{array} \right.$

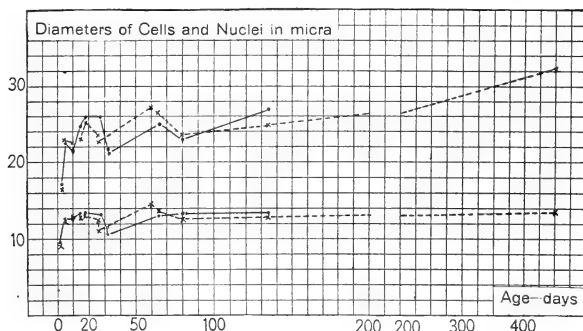


Chart 3 Based on table 3 and giving the computed diameters of the cells and their nuclei according to sex—on age in days. Wild Norway rat. The values for one female at 450 days are given in the chart. This case is not entered in table 3. At 200 days, as indicated by a break in the graph, the time unit is changed—one division being made equal to twenty-five days instead of ten days as heretofore. Males ——— Females -----

To return to table 3, the ratios between the cell and nucleus tend to increase up to the age of twenty-five days; from that time on the ratio in every case is almost 1:1.9. It should be noticed, moreover, that there is during this later period no increase in the ratios as the animal increases in age or in size. This fact will be discussed later on.

TABLE 5

Giving according to percentage of water in the brain the computed diameters of the cells and nuclei in the superior cervical ganglion of the Norway rat. Sexes separated. Data condensed

MALE					FEMALE				
Mean body weights	Number of cases	Percentage of water in brain, average	Computed diameter		Computed diameter		Percentage of water in brain, average	Number of cases	Mean body weights
			Nucleus	Cell	Cell	Nucleus			
			μ	μ	μ	μ			
100	1	80.4	13.4	26.0	25.5	12.7	80.4	2	68
54	2	79.5	12.3	24.2	23.7	12.6	79.6	5	46
153	5	78.7	13.3	28.4	26.6	13.0	78.7	4	129
172	5	78.3	13.3	28.2	27.2	13.1	78.5	5	164
258	7	78.1	14.1	32.4	29.5	13.5	78.2	5	203
194	2	77.7	13.0	28.9	30.2	13.5	77.8	2	205
253	3	77.4	14.1	31.4	26.6	12.5	77.4	2	175
331	7	76.8	13.5	30.8	28.2	12.8	77.0	5	203

D. In relation to the percentage of water in the brain as an indication of age

Accepting the conclusion (Donaldson, '10) that the percentage of water in the central nervous system is more closely correlated with age than with body weight and brain weight, it was thought worth while to study the growth of these cells in relation to the percentage of water in the brain, in order to supplement what has been presented in the preceding paragraph, based on animals of known ages.

Table 5 is, therefore, to a certain extent, a continuation of table 3. For each sex there are eight entries based on the condensed data, those cases with known ages being excluded. The corresponding graphs appear in chart 4. According to Don-

aldson ('11) the percentage of water in the central nervous system of the Albino and Norway rat at like ages is nearly the same, so the ages of the Norway rats whose percentages of water are known may be obtained from table 74 of 'The Rat' (Donaldson, '15) and the growth of the cells as shown in chart 4 can be translated into age. Using this procedure, the curves in chart 4 represent the gradual growth from twenty-five days of age to maturity.

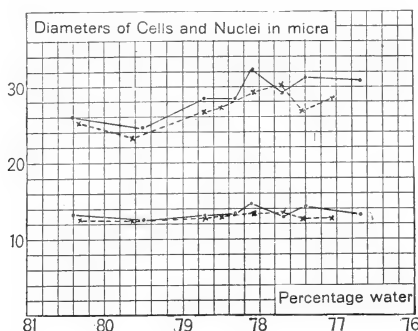


Chart 4 Based on table 5 and giving the computed diameters of the cells and their nuclei according to sex—on percentage of water in the brain. Wild Norway rat. Males ——— Females -----

The examination of table 5 enables us to see that in general the female has a slightly higher percentage of water in the brain than the male, due probably to the smaller absolute size of the brain (Donaldson, '16), but the cells of the male exceed in diameter those of the female in seven out of eight cases.

MORPHOLOGY OF THE LARGE CELLS

The general morphological changes in the large cells from birth to maturity are similar to those in the cells of the Albino. The large cells of the first few days are much alike in the two forms. The distribution of Nissl granules and the tendency to accumulate at different regions in the cell in the later ages are also alike in the two forms. There is, however, some difference

between the Albino and the Norway rat in regard to the degree of aggregation of the Nissl bodies in the cells. Thus, in the cells of the Norway, these are not so densely crowded either at the periphery or around the nucleus as in the cells of the Albino.

Furthermore, the space left at one region through the crowding of Nissl bodies toward another is not so clear. There is therefore always found a gradual thinning out of the granules toward the periphery or the nucleus whenever the accumulation of them takes place in a reverse direction.

When the Norway rat reaches twenty days of age, we find in the cells of the superior cervical ganglion a tendency for the Nissl bodies to aggregate at the periphery, though the region around the nucleus is by no means devoid of them. Likewise, when the Norway rat is about sixty or more days old, the Nissl bodies tend to accumulate around the nucleus, while the periphery still has some of them thinly scattered. Unless carefully examined, therefore, the distribution of the Nissl bodies in the cells at twenty to sixty days and in those sixty days and later would appear the same, i.e., as if they were evenly distributed.

Like the Albino, the distribution of the granules in the cells of the older Norways is fairly even. On the whole, then, there is no marked distinction between the two forms, so far as the general morphology of these cells is concerned.

According to Gaskell ('18), both the motor and the inhibitory cells are found in the sympathetic ganglion, and Cajal ('11), using the Golgi method, shows in the superior cervical ganglion of a mouse several days old cells which appear to represent three different types (fig. 550, B, C, and D). That the four types of cells (Ping, '21) found in the superior cervical ganglion of both the Albino and Norway rat are correlated with the several functions of the ganglion is by no means determined, but, generally speaking, we should expect that the morphological distinctions would have a functional significance.

Binuclear cells

The number of the binuclear cells in one ganglion in each case is recorded in table 6. Generally speaking, we may say that this type of cell is found through the whole span of life.

TABLE 6

Norway rat: Data for eighty individuals arranged in eighteen groups according to the decreasing value of the percentage of water in the brain. This is indicative of increasing age. Under the 'number of cells' the number of any class of cells in one ganglion of each individual in which such cells occur is given. Thus in the first group one individuals showed three binuclear cells, but no pigmented or vacuolated cells were found. In the fifteenth group binuclear cells were found in four out of the five individuals, pigmented cells in four, and vacuolated cells in one

NUMBER OF CASES			AVERAGE PERCENTAGE OF WATER IN THE BRAIN	AVERAGE BODY WEIGHT	NUMBER OF CELLS IN INDIVIDUAL CASES—ZEROS OMITTED		
M.	F.	Total			Binuclear	Pigmented	Vacuolated
				grams			
1	2	3	88.33	11	3	—	—
2	1	3	87.98	11	1	—	—
2	1	3	84.42	21	3, 3	—	—
—	3	3	81.45	29	1, 1	—	—
2	2	4	80.30	67	1	—	—
1	3	4	79.83	40	1, 1, 1	—	—
3	1	4	79.43	46	1, 1, 1	—	—
3	2	5	78.87	119	1, 5, 8, 3	—	—
2	3	5	78.69	120	1, 5, 3, 3, 1	2	—
1	4	5	78.47	183	5, 9, 2, 4	1	—
3	2	5	78.41	123	3, 1	—	—
4	1	5	78.29	201	2, 2, 4, 14	2, 13	—
2	3	5	78.14	220	5, 2, 10, 1	1, 3	3
3	2	5	78.01	236	7, 3	1, 3, 1	3
4	1	5	77.60	240	2, 2, 10, 3	9, 3, 15, 4	1
1	4	5	77.25	189	5, 2, 4, 2	85, 3, 2, 6	—
2	3	5	77.00	279	2, 1, 1, 2, 2	1, 2, 5	—
5	1	6	76.66	307	9, 3, 3, 4	2, 5, 1, 37, 15, 1	1

There are, however, exceptions, since, as the table shows, this type of cell is present in all the individuals composing a group in two instances only.

As can be seen, binuclear cells appear usually in very small number and frequently only one is found in the entire ganglion,

and the maximum number of these cells is never above ten. There seems to be a tendency for the binuclear cells to increase in number during the middle age of the animal. The diameters of some of these cells and those of their two nuclei, as well as the nucleus-plasma ratios derived from these diameters, are recorded in table 7.

According to table 7, the nucleus-plasma relation is 1:4.6 in a very young rat and 1:4.1 in an old rat, as indicated by the body weight, and throughout the series the values are fairly constant, though with a slight tendency to diminish. This agrees with what has been found in the Albino and shows that

TABLE 7

Diameters of some of the binuclear cells and of the two nuclei in each of them. Data arranged according to body weight. The nucleus-plasma ratios are shown in the last column. Norway rat

BODY WEIGHT	DIAMETER OF		RATIO OF VOLUME OF CYTOPLASM TO VOLUME OF TWO NUCLEI
	Cell	Two nuclei	
<i>grams</i>	μ	μ	
13.6	25.0	11.6 + 10.7	1 : 4.6
33.0	29.9	13.4 + 13.4	1 : 4.6
115.0	26.3	13.4 + 8.9	1 : 4.8
169.2	28.1	13.4 + 12.9	1 : 3.9
243.2	27.7	12.0 + 13.4	1 : 4.1
230.2	28.1	13.4 + 13.4	1 : 3.6

in the binuclear cells there is an increase in the nuclear mass which is not accompanied by the same enlargement of the cytoplasm, as is found in mononuclear cells. In their nucleus-plasma relation, therefore, these binuclear cells are like very young cells, and in the older animals at least they certainly do not represent two normal mononuclear cells pressed together.

Pigmented cells

It is somewhat surprising to find that the pigmented cells in the superior cervical ganglion of the Norway are not very numerous, as table 6 shows. In no case were they found in all of the individuals of a group. They do not appear in the young

animal, the first being found in my series at a body weight of 120 grams. In this case the percentage of water in the brain was 78.69, which corresponds to the age of eighty-eight days—this happens to be exactly the age at which they first appeared in the Albino. The number of the pigmented cells tends to increase as the animal grows older. There are two cases among the older rats which show large numbers of pigmented cells, but most ganglia have only a few, even at the later ages. There is less increase in the number of these cells in the Norway, as contrasted with the Albino, than we should have expected. The pigment granules are black or greenish black in color. It was a matter of some surprise to find that in the gray pigmented rat the cells contained hardly more pigment than appeared in the Albino.

Vacuolization of the cells

Incidentally vacuoles have been noted in a very few of the cells in the superior cervical ganglion of the Norway, although none were observed in the Albino. Out of eighty cases only eight showed vacuoles in the cells and in an entire ganglion only one to three vacuolated cells have been found (table 6). Most of these cells have but one vacuole, which is oval in shape, and which may lie close either to the nucleus or to the periphery, but in one cell two vacuoles were found. The size of the vacuole varies; generally it is smaller than the nucleus, rarely larger. It resembles the nucleus in outline, but, owing to the absence of any internal structure, can be recognized without difficulty. All the vacuolated cells were found in older rats.

Increase in the number of the large cells

In counting the large cells found during the first twenty-five days, I followed the procedure previously used for the Albino. The cells of the Norway are small as compared with those of the Albino, especially at birth, so I have extended the limiting values, 19 to 25 μ , which were used for the diameters in counting the large cells of the Albino, to 16 to 25 μ for the Norway.

Table 8 gives the number of these large cells recorded in the ganglion for each sex during the first twenty-five days.

There are a few advanced cells and a few comparatively large cells at birth, or just after, but the number is strikingly small. By the end of the fifth day there is a great increase in number—about twenty times that of the preceding stage. Then the

TABLE 8

Increase in number of large and advanced cells, 16 to 25 μ in diameter, during the first twenty-five days of age. The ratios for the increase in the total number for both sexes between one day and nineteen days stand at the foot of the last column. Superior cervical sympathetic ganglion. Norway rat

SEX	AGE	BODY WEIGHT	NUMBER OF LARGE CELLS
	<i>days</i>	<i>grams</i>	
♂	1	5.9	22
♀	1	5.6	24
♂	5	12.7	416
♀	5	13.4	515
♂	10	13.6	417
♀	10	13.5	530
♂	15	17.6	833
♀	15	13.6	827
♂	19	31.1	3,066
♀	19	31.1	3,084
♀	25	28.5	3,074
Ratios between 1 and 19 days.....			$\left\{ \begin{array}{l} \text{♂} \quad 1 : 139.3 \\ \text{♀} \quad 1 : 129.0 \end{array} \right.$

increase is slow and slight until the age of fifteen days. At this time the cells again show a considerable increase in their number, and this is still more marked at nineteen and twenty-five days. Thus the greatest increase in number of the large cells is between one and five days, and again between fifteen and nineteen as in the case of the Albino (Ping, '21, table 6).

The ratio of increase is a trifle higher for the male Norway, as was found for the male Albino; indeed, the relations of the ratios according to sex are strikingly similar in the two strains.

As will be seen by comparing the ratios (1 to 19 days) for the Norway with those (1 to 20 days) for the Albino, the rate of increase is apparently ten times as great in the Norway as in the Albino. This will be discussed later.

The nucleus-plasma relation

In determining the increase in volume of the cytoplasm in relation to that of the nucleus, the computed diameters of the

TABLE 9

Giving the average diameters of the cells and nuclei according to body weight. The nucleus-plasma ratios based on these diameters are given in the last column. Data condensed. Superior cervical sympathetic ganglion. Norway rat

NUMBER OF CASES	BODY WEIGHT RANGE	MEAN BODY WEIGHT	DIAMETERS		NUCLEUS-PLASMA RATIOS
			Cell	Nucleus	
		<i>grams</i>	μ	μ	
16	6-38	22.2	22.3	12.1	1 : 5.3
16	41-100	65.9	25.0	12.8	1 : 6.4
8	104-150	121.4	26.8	13.0	1 : 7.7
16	152-195	175.4	27.9	13.1	1 : 8.6
16	206-250	226.6	30.1	13.5	1 : 10.0
5	259-290	270.1	32.7	13.7	1 : 12.4
8	311-402	346.1	32.2	13.8	1 : 11.6

cell and of the nucleus have been condensed according to body weight and arranged in table 9. By subtracting the volume of the nucleus from that of the cell, the volume of the cytoplasm is obtained, and the ratios between the latter and the volume of the nucleus are given in the last column of table 9. This table shows that the increase of the cytoplasm is progressive, except in the last group.

In general it may be said that for each 50 grams of increase in body weight, the increase in the ratio is one unit. For a body weight of 22.2 grams (about twenty-five days of age) the ratio is about one-half that for the oldest group with a body

weight of 346 grams. As compared with ratios for standard Albinos of like body weights (table 2, Ping '21), the ratios for the Norway are clearly low.

DISCUSSION

In the foregoing paragraphs the growth of the cells in the Norway rat has been treated in relation to the body weight and length and in relation to age, either observed or inferred from the percentage of water in the brain of the animal. In each case the results show that the growth is comparatively rapid at first and then becomes gradual. Moreover, as was to be expected, the growth of the sympathetic nerve cells of the Norway resembles in a general way that of the standard Albino. There are, however, differences between these forms, worthy of note, and to make the comparison as complete as possible, the data for the inbreds will also be taken into consideration.

Although the form of these data and the numbers of cases are not the same in the three series, they are yet sufficiently similar to make several comparisons worth while.

Before attempting this, a word about the general relations of the three strains here examined is in place. Both the Albino strains contained animals which had been in captivity for many generations, and were also domesticated in the sense that they had lost the fear of man and were easy to handle. The Norway strain, on the other hand, had two groups in it: (1) the rats caught wild and of unknown age—represented in this series by animals 37 grams or more in body weight—and (2) a group which were the F_1 or F_2 descendants of Norway parents caught wild. Although this second group was composed of captive individuals, they were by no means domesticated and for the most part were still timid and excitable.

The differences between these strains may be tabulated as follows:

<i>Albinos</i>	<i>Norways</i>
Not pigmented	Pigmented
Captive	Wild or captive
Domesticated	Not domesticated
(A) 'Standard' (not inbred)	Not inbred
(B) 'Inbred'	

By the aid of such a tabulation, there seems to be a chance to consider the possible influence of albinism, captivity, domestication, and inbreeding on the cells under discussion.

The characters which may be compared in the several strains are:

A. The morphology of the largest cells.

B. Special cell forms (binuclear, pigmented, or vacuolated).

C. The increase in the diameter of the cells from birth to maturity.

D. The absolute size of the cells at different ages.

E. The nucleus-plasma ratios.

F. The rate of the formation of large from small cells.

In making the comparison, the three strains will be briefly designated as 'standards,' 'inbreds,' and 'Norway,' and for convenience the values for the 'standards'—which have been most completely studied—will be those to which the values for the other strains are referred.

A. The morphology of the largest cells

Figures 1 to 5 (Ping, '21) show the morphology of the largest cells in the standards. In the other strains these cells have in general a similar appearance. However, it was noted in the Norways that the Nissl granules were less segregated than in the standards. Whether this difference is correlated with albinism or domestication cannot be determined at present, because the Norways have not yet been domesticated.

B. Special cell forms

1. *Binuclear cells.* In the standards, binuclear cells were found in every ganglion examined (table 5, Ping, '21) and the average number was 4.2 per ganglion. In the Norway they were found in only 54 out of 80 ganglia studied (table 6). The average number for the entire series of 80 ganglia was 2.2 per ganglion, and for the 54 ganglia in which they occurred, 3.3. In the Norways therefore, binuclear cells are less abundant. Again this difference cannot be correlated with either albinism or domestication.

TABLE 10

Increase from birth to maturity. The data for the 'standards' are from table 1 (Ping, '21) and for the Norways from table 1 of the present paper. In each instance the values are the means for the male and female records at the corresponding body weights

BODY WEIGHT		DIAMETERS	
		Cell	Nucleus
Standards	5.9	μ 19.7	μ 10.8
	178.0	30.5	13.5
Ratios.....		1.54	1.25
Percentage increase.....		55	25
Norways	5.8	16.9	9.7
	182.0	27.8	13.2
Ratios.....		1.64	1.36
Percentage increase.....		64	36

TABLE 11

Showing, in the standard Albino and in the Norway rat at about 19 grams and 178 grams of body weight, the diameters in μ of the largest cells and nuclei in the superior cervical sympathetic ganglion, compared with those of the cells from the ventral horn of the spinal cord (seventh cervical segment) and from the corresponding ganglion (Donaldson and Nagasaka, '18)

LOCALITY	STRAIN		AT 19 GRAMS	AT 178 GRAMS	GAIN	
					Absolute	Percentage
Superior cervical sympathetic ganglion.....	Standard	Cells	24.2	30.5	6.3	26
		Nuclei	12.1	13.5	1.4	12
Motor cells, spinal cord....	Standard	Cells	23.9	29.1	5.2	22
		Nuclei	12.9	15.1	2.2	17
Spinal ganglion cells.....	Standard	Cells	21.6	34.2	12.6	58
		Nuclei	10.6	16.4	5.8	55
Superior cervical sympathetic ganglion.....	Norways	AT 23 GRAMS		AT 182 GRAMS		
		Cells	23.0	27.8	4.8	21
		Nuclei	12.4	13.2	0.8	6

2. *Pigmented cells.* In the standards the pigmented cells do not occur before puberty (table 5, Ping, '21); the same is true for the Norways (table 6). In the standards there were found about 3.4 pigmented cells in each of the eleven ganglia in which such cells occurred, while in the Norways there were 3.8 pigmented cells per ganglion, after these cells were first noted at 120 grams of body weight.

However, in one ganglion of the Norways eighty-five, and in another thirty-seven pigmented cells were found, and it is these two cases which make the average for the Norways slightly above that for the standards. This is a surprising result, for, according to common teaching, we should have expected a much greater number of pigmented cells in the Norways than in the standards. Until thoroughly domesticated Norways are available, these relations cannot be interpreted.

3. *Vacuolated cells.* These vacuolated cells were not found in the standards, but do occur in the Norways after maturity. Their number is small (table 6). As the data stand, the vacuolated cells are characteristic for the mature wild Norways.

C. The increase in the diameters of the cells from birth to maturity

As between the standards and Norways, it is possible to make this comparison between birth and maturity; table 10, gives what has been found.

As the ratios and percentages show, the cells and nuclei have increased a little more in diameter in the Norway than in the standards, but this difference appears to depend mainly on the small size of these cells at one day of age in the Norway.

If we choose the values at about 19 grams of body weight for the initial data, it is possible to compare the changes in the diameters of these cells, not only in the standards and Norways, but also with those of the cells in the spinal ganglia and ventral horn of the spinal cord, as observed in the standard rats (Donaldson and Nagasaka, '18). The relations appear in table 11.

This table 11 may be used for two purposes. First, it shows that within the limits of body weight chosen the percentage

gains in these cells are of the same order in the Norways as in the standards—21 per cent and 26 per cent, respectively, for the cell body. The two strains are therefore similar in this character. Second, that this order of enlargement is, on the one hand, similar to that found for the large motor cells in the spinal cord, and, on the other, very different from that found for the corresponding spinal ganglion cells. This later relation indicates that the sympathetic ganglion cells, which are efferent in function, behave like motor cord cells during their later growth.

TABLE 12

Comparing, according to body weight, the average diameters of the cells and nuclei in the superior cervical sympathetic ganglion of the Norway with that of the standards. The data for the Norway rat are condensed from table 2, and for the Albino from table 2 of the former study (Ping, '21). Sexes combined

ALBINO RAT			NORWAY RAT		
Body weight	Nucleus	Cell	Cell	Nucleus	Body weight
<i>grams</i>	μ	μ	μ	μ	<i>grams</i>
6	10.8	19.7	16.8	9.7	6
15	13.1	25.7	22.6	12.7	14
31	12.2	25.0	23.9	12.4	32
53	13.2	27.2	24.3	12.7	51
73	13.2	29.2	25.8	13.0	74
106	13.6	29.8	25.7	13.0	103
151	13.2	28.9	27.2	13.3	157
178	13.5	30.5	27.2	13.1	189
Average ..	12.8	27.0	24.2	12.5	

Percentage difference in cell diameter = 11 per cent

Percentage difference in nucleus diameter = 3 per cent

D. Absolute size of cells at different ages

E. Nucleus-plasma ratios

In the first instance we shall take up cell size alone and limit the comparison to that of the Norways to the standards.

In table 12 the mean diameters of the cells and nuclei of the standards and Norways are arranged according to their respective body weights, the former data being from table 2 of my previous paper, the latter from the foregoing table 2 of the present paper.

A study of table 12 gives a clear idea of the quantitative difference in growth between these two forms, and the same data are represented by graphs in chart 5. From the chart it is evident that the diameters of the cell body in the Norways are consistently below those in the standards.

The data for these two strains have been compared also on body length, on age, and on the percentage of water in the brain and by all of these methods of comparison show relations essentially like those just presented. Thus, no matter what the basis of

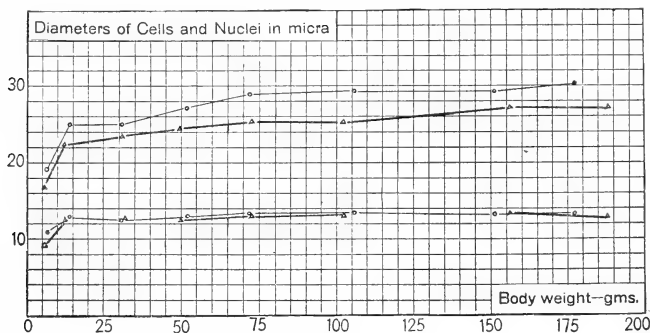


Chart 5. Based on table 12 giving the diameters of the cells and their nuclei on body weight in the Norway rat compared with the standard albino.

Norway $\Delta - \Delta$ Albino $O - O$

comparison, these cells in the Norways are smaller than those in the standards.

In order to compare with the standards the inbreds as well as the Norways, a series of data, selected from the previous records, have been assembled in table 13.

An examination of table 13 brings to light several interesting relations. While in five out of the six instances the diameters of the nuclei are similar, there is nevertheless a great difference in the diameters of the cells according to strain. The differences therefore appear mainly in the cytoplasm. As compared with the standards, the inbred cells are small, while the cells of the Norways, though also small, differ much less.

The smaller size of the cells in the inbreds and Norways is not related to pigmentation, for the inbreds with the smaller cells are not pigmented. It is not due to domestication, for both the standards and inbreds are domesticated.

It would appear therefore to be a characteristic of this inbred strain, but without further evidence it cannot be said to be due to inbreeding.

Since the differences according to strain are mainly in the cell, the nucleus-plasma ratios show the same relations as do the cell diameters, and the most striking feature is the small value

TABLE 13

Giving the diameters and nucleus-plasma ratios of the standard, inbred and Norway rats according to body weight, based on previous tables. In each instance the values given are for the two sexes combined

STRAIN	BODY WEIGHT	DIAMETERS		NUCLEUS-PLASMA RATIO
		Cells	Nucleus	
	<i>grams</i>	μ	μ	
Standard.....	145	28.9	13.2	1 : 9.5
Inbred.....	144	24.5	13.2	1 : 5.4
Norway.....	146	28.0	13.1	1 : 8.8
Standard.....	200	34.1	14.5	1 : 12.0
Inbred.....	206	25.4	13.3	1 : 5.9
Norway.....	203	28.0	13.1	1 : 8.8

for the ratios in the inbreds. The data for the heavier body weights show that in this group these cells in the inbreds have only half the volume of those in the standards.

Perhaps the point of most general interest which is thus brought out is the plasticity or variability in the size of this group of cells according to strain.

F. The rate of the formation of large from small cells

As table 6 (Ping, '21) shows, in the standards the number of large and advanced cells increases about thirteen-fold from one to twenty days. On the other hand, table 8 of this paper indi-

cates in the Norway an increase during this same interval, which is about 135-fold, or nearly ten times as large. One circumstance which contributes to this result is the lower standard which was taken for the large cells in the Norway, in order to get a reasonable number of such cells at birth. The use of this lower standard gives of course a higher number at maturity in the Norway. This is, however, a trifling matter, and the more important difference between the two strains lies in the relatively small number of large cells in the Norway at one day of age.

This is an expression of retardation in the early growth of the Norway in captivity—a peculiarity which has been noted by several observers and which has been demonstrated by Dr. Helen D. King for the general body growth.

Here again it is not possible to offer a precise explanation, for this relative retardation may be a character of the Norway in the wild state and hence difficult to study, or it may be a response to captivity, as these observations were made on young born in captivity from Norways not yet domesticated.

It is possible, however, to conclude that the retardation in the early development of these cells is found in the wild as contrasted with the domesticated standard strain.

To determine the general significance of these results, a brief review of some earlier observations along similar lines may be of value.

In the presentation of these the data for the wild Norway will be taken as the standard, as this is the strain from which the Albino has been derived.

As compared with wild Norways of like body weights, the standard Albinos have lighter brains and lighter spinal cords (16 and 12 per cent, respectively; Donaldson and Hatai, '11). Further, two portions of the brain, the olfactory bulbs and the paraflocculi, are, relatively, still lighter in the Albino.

As between the sexes within the same strain, it was found that while the weight of the spinal cord (on body weight) was similar in the two sexes in the Norways, yet in the standard Albinos the weight of the cord in the female was relatively heavier than in the male (Donaldson and Hatai, '11).

On comparing the diameters of the cells in the cerebral cortex of the rat, Sugita ('18) found that those in the lamina pyramidalis and in the lamina ganglionaris of the Albinos were, respectively, 4 and 7 per cent less in diameter than the corresponding cells in the Norway.

In the foregoing instances it appears that there is a reduction in the relative size of the parts of the nervous system, as well as in some of the cell elements, in the Albino as compared with Norway.

Incidental observations on captive Norways suggest that this reduction is the result of captivity or domestication. There remains, however, the difference in the relative weights of the spinal cord which occurs in the Albino, but is absent in the Norway, and in this instance it is possible that albinism plays a part. From these earlier observations we should have expected to find the cells in the superior cervical sympathetic ganglion smaller in the Albinos.

Contrary to expectation, these cells in the standards are larger than in the Norway, but also exhibit a sex difference, being larger in the females. Nevertheless, in the case of the inbreds, these cells are much smaller than in the Norways, and show only a slight difference according to sex. At the moment, therefore, the size of these cells in the standards does not agree with the expectation based on the results obtained from other parts of the nervous system, but any simple interpretation is at once precluded by the very small size which they have in the inbreds.

SUMMARY

1. The growth of the largest cells in the superior cervical sympathetic ganglion of the Norway has two phases: (1) a comparatively rapid growth from birth to twenty-five days, (2) followed by a slow and gradual growth continuing to the end of the record.

2. The growth of these cells in relation to body weight, body length, age, and the percentage of water in the brain does not show differences due to sex after the animal has passed puberty.

3. The morphological changes in the cells are in general similar to those found in the Albino, but the stainable substance is somewhat less aggregated. The binuclear cells and the cells with pigment are present, as in the Albino, but the latter are only slightly more numerous in the Norway. Besides, there are in the Norway a few vacuolated cells.

4. The mode of growth of the cells, as in the Albino, shows similarity to that of the motor cells of the spinal cord.

5. The nucleus-plasma ratio is 1 to 5 at birth and 1 to 12 after maturity.

6. The cells at birth are about 16 per cent less in diameter than those of the Albino. The growth acquired after the weaning period is about 10 per cent less than that of the Albino, such difference being probably due to domestication.

7. Comparing with the standard Albino the data for the wild Norways, it appears that in the Norways the diameters of the nuclei are the same, but those of the cell bodies are slightly less. As a consequence, the nucleus-plasma ratios are smaller in the Norway. Moreover, there is no clear sex difference in the size of these cells.

8. Comparing the inbreds with the standard albinos in respect of these cells, it appears that in the inbreds the sex difference in size is less marked. The absolute diameters of the nuclei are similar to those for the standards and Norways, but the diameters of the cells are much less, and in consequence the nucleus-plasma ratios are only about half those for the standards.

9. The interpretation of these differences awaits the data to be obtained from Norways after long-continued domestication and inbreeding.

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Resumen por el autor, Howard Ayers.

Cefalogénesis de los Vertebrados.

V. Origen del aparato mandibular y del complejo del trigémino en *Amphioxus*, *Ammocoetes*, *Bdellostoma* y *Callorhynchus*.

Las mandíbulas de los Vertebrados presentan una larga historia de transformaciones y adaptación. Han comenzado con el aparato mandibular del *Amphioxus*, que contiene solamente el elemento mandibular de las formás superiores más otras estructuras que desaparecen en los peces cartilaginosos, en los cuales aparecen bajo forma de cartílagos labiales. El ejemplo mejor conservado de mandíbula del tipo del *Amphioxus* entre los gnatostómos se encuentra en *Callorhynchus*. Su mayor desarrollo se alcanza en *Bdellostoma*. El mecanismo hioideo se desarrolla en forma de una estructura de soporte a lo largo del mecanismo mandibular, mucho antes de aparecer los elementos maxilares, y experimenta una regresión al mismo tiempo que los llamados cartílagos labiales y en este caso, de nuevo, su sección anterior se conserva mejor entre los gnatostómos en *Callorhynchus*.

Mientras que la historia del elemento mandibular de la mandíbula de los gnatostómos es completa en general, el curso de la evolución del esqueleto del elemento maxilar necesita todavía trazarse. El aparato mandibular ha precedido a las barras branquiales, y por consiguiente, no está genéticamente relacionado con ellas. El nervio trigémino se origina en *Amphioxus* como un grupo de cinco nervios segmentarios que inervan el aparato mandibular y ha sufrido una condensación de sus partes hasta que en los gnatostómos aparece como un solo tronco nervioso complejo. En *Bdellostoma* los cinco nervios ancestrales que le componen pueden todavía distinguirse.

VERTEBRATE CEPHALOGENESIS

V. ORIGIN OF JAW APPARATUS AND TRIGEMINUS COMPLEX— AMPHIOXUS, AMMOCOETES, BDELLOSTOMA, CALLORHYNCHUS

HOWARD AYERS

THIRTY-SIX FIGURES

The current theory of the origin of the vertebrate jaws assumes their derivation and descent from a pair of gills, with all the structural implications involved in that assumption. The food canal antedated gills, mere appendages of the wall of the food canal. The anterior and posterior openings of the canal undoubtedly antedated not respiration, but the complicated branchial respiration of vertebrates. Branchial respiration in these forms is carried on by means of the richly vascular walls of holes through the gut and body walls.

The phylogeny of these structures is insufficiently indicated by their ontogeny in the lower vertebrates, and comparative anatomy has not as yet revealed the story of their descent. In any event, the armature of the anterior opening of the food canal antedated the highly specialized gills of vertebrates as we yet know them, and all the evidence we have on this subject indicates that the jaws are in no way genetically related to branchial structures of any kind.

The testimony of *Amphioxus*, *Ammocoetes*, *Bdellostoma*, and *Callorhynchus*, bearing on this subject, is here presented for the purpose of solving a few of the host of questions which are involved in the phylogeny of the vertebrate jaws. Two conclusions that the testimony fully sustains are: 1) That the jaw apparatus is in no way related to gills, but is genetically related in the forms mentioned in the order named, and since *Callorhynchus* is an accepted Gnathostome, it follows that the *Amphioxine* jaw apparatus is the earliest known condition of

the gnathostome jaw. 2) That the jaw apparatus is the end-organ of the trigeminus. These two conclusions are presented before the evidence, in order that the reader may better weigh the evidence given.

In the past the study of the jaw began with the examination of the human jaw and worked down through the long series of vertebrate forms, seeking evidence to homologize and explain the origin and transformation of these parts in the vertebrate stock. Embryological evidence was abundantly used to check the results and to clarify the relationships. A theory of the origin of the jaw was thus worked out, and it became generally accepted among morphologists that the jaw was derived from one of the anterior gills, presumably the first pair of the ancestral vertebrate. However, indications of presumptive premandibular structures were found which cast doubt on the accuracy of this conclusion as regards the particular pair of gills involved.

The Myxinoids could not be brought into this arrangement because they were held to have no jaws. They were set aside in a special group—Cyclostomes—and contrasted with the Gnathostomes. Amphioxus was considered to have nothing even suggesting jaws.

The jaw apparatus plays such an important rôle in cephalization of the vertebrate body that I have for quite some time given special attention to the subject, particularly to what Amphioxus, Ammocoetes, and Bdellostoma have to tell, and I think they give a clear, straight story of how the jaw came to be.

The material used in this study consisted: 1) of adult and young stages of Amphioxus from the Naples Station treated with different reagents. 2) Ammocoetes and Petromyzon from Cayuga Lake, consisting of a series of stages covering the whole Ammocoetes period, stages illustrative of the metamorphosis and heads of the adult Petromyzon. It is a pleasure to acknowledge my indebtedness to Prof. Simon H. Gage, of Cornell University, for this adequate material, which was of great value in my investigation. I am particularly grateful to Professor Gage for his generosity in supplying me with Ammocoetes undergoing metamorphic changes. 3) Bdellostoma from the Bay of Mon-

terey, partly collected by myself and partly by Miss Julia Worthington, who generously placed her extensive collections including adults, larvae, and embryos at my disposal. 4) *Bdellostomas* from Cape of Good Hope, *Myxine* from Norway and Torres Straits, *Hexanchus* from Bay of Monterey, various *Elasmo-* branches from Woods Hole and Trieste, *Callorhynchus*, *Chimaera*, several species of *Urodeles* both larval and adult, and several species of *Anura* both larval and adult.

A discussion of the literature is reserved for subsequent publication.

AMPHIOXUS

Under the term jaw apparatus are included all the structures constituting the cirri or tentacular apparatus of *Amphioxus* as recorded in the literature of the lancelet—skeleton, muscles, nerves, etc.

The jaw bars form the internal skeleton of the armature of the buccal aperture of the oral hood. They are two elastic chondral bars, one in the left lip, the other in the right lip of the mouth. Each bar is composed of from seventeen to twenty segments progressively smaller from the base behind to the terminal segment in front. Each segment is prolonged into a tapering filament or tentacle which projects from the distal end of each segment.

The two jaw bars are bilaterally symmetrical and, seen from above, curve outward from the median line in a gentle curve, until about midway of their course, when they curve inward so that the tips lie side by side in the anterior pocket of the buccal cavity (fig. 1). Seen from the side, the bars curve dorsad from the base and then ventrad, then dorsad again to form an open S-shaped figure. These curves have their part in the functioning of the jaw apparatus in opening and closing the mouth. Under varying pull of the muscles, the bars change their shape.

Thus the bases of the bars lie in the ventral wall of the body and the tips lie close under the notochord. The buccal aperture, thus looks forward and downward; it is placed at an angle to

ABBREVIATIONS

<i>A</i> , anastomosis	<i>Nv</i> , velar nerve
<i>B</i> , buccal cavity	<i>O</i> , oral hood
<i>b</i> , attachment of jaw bar to hyoid	<i>ol</i> , ophthalmic lateralis nerve
<i>Br</i> , brain	<i>on</i> , ophthalmic nerve
<i>C</i> , cornual cartilage	<i>oph</i> , ophthalmic nerve
<i>D</i> , skin	<i>os</i> , oral sphincter
<i>d</i> , dorsal spinal nerve	<i>P</i> , palatine
<i>E</i> , endostyle	<i>Pc</i> , palato-coronarius muscle
<i>Ei</i> , eye	<i>Pl</i> , plexus of jaw apparatus
<i>G</i> , gills	<i>Q</i> , quadrate
<i>H</i> , hyoid	<i>R</i> , right side
<i>H'</i> , hyoidean procartilage	<i>r</i> , right median tentacle
<i>Hs</i> , hyoid arch	<i>Rm</i> , rectus muscle
<i>Hy</i> , hypophysial canal	<i>S</i> , subnasal bar
<i>J</i> , jaw bar	<i>Se</i> , septalis nerve
<i>Jd</i> , dentigerous jaw cartilage	<i>sm</i> , myoseptum
<i>Jl</i> , jaw ligament	<i>sp</i> , occipitospinal nerves
<i>Jo</i> , median ventral jaw bar	<i>T</i> , tentacle
<i>L</i> , left side	<i>t</i> , thyroid
<i>l</i> , left median tentacle	<i>Te</i> , terminalis nerve
<i>li</i> , ligament	<i>Tm</i> , median tentacle of Ammocoetes
<i>M</i> , myotome	<i>U</i> , upper lip
<i>mc</i> , circular velar muscle	<i>Ul</i> , lower lip
<i>mn</i> , nasalis muscle	<i>V</i> , velum
<i>Me</i> , endostyle muscle	<i>v</i> , ventral spinal nerve
<i>Md</i> , mandibular nerve	<i>vc</i> , velar chamber
<i>Mj</i> , jaw muscle	<i>Vl</i> , velar ligament
<i>Mn</i> , ventral transverse muscle	<i>Vn</i> , velar nerve
<i>mr</i> , radial velar muscle	<i>vq</i> , veloquadratus muscle
<i>mv</i> , velar muscle	<i>W</i> , wall of oral hood
<i>Mx</i> , maxillary nerve	<i>I to V</i> , jaw muscles of Amphioxus
<i>N</i> , nasalis nerve	<i>VII</i> , facial nerve
<i>n</i> , nerve	<i>VIII, a and b</i> , two acusticus nerves to snout complex
<i>Na</i> , nose	<i>IX</i> , glossopharyngus nerve
<i>Nc</i> , notochord	<i>X</i> , vagus nerve
<i>Nl</i> , nasal capsule	
<i>No</i> , nasal canal	

Figs. 1 to 13, except fig. 10, *Amphioxus*.

Figs. 14 to 21, *Ammocoetes*.

Figs. 22 to 36, except fig. 30, *Bdellostoma*.

Fig. 30, *Chimaera*.

Fig. 10, *Callorhynchus*.

the long axis of the body. This angle is greater in both *Ammo-coetes* and *Bdellostoma* than it is in *Amphioxus*.

The jaw bars extend the entire length of the buccal aperture, which is not quite as long as the buccal chamber, owing to the pocket which lies behind the base of the jaw bars and ends in front of the velum. The bases of the jaw bars are tied together by tendons and muscle.

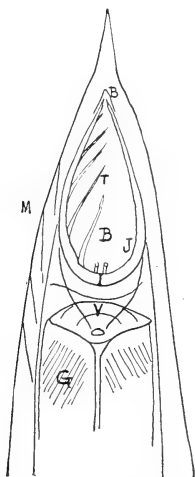


Fig. 1 Ventral view of the anterior end of *Amphioxus* to show relation of the jaw apparatus to the body wall, myotomes, and velum.

The extension of the muscles forward to the tips of the bars and out onto the tentacular projections ties all skeletal parts into a unit structure for the work it has to do.

The chondral jaw bars which frame the buccal opening in *Amphioxus* are the simplest expression among living forms of the vertebrate mandible. But they include much more than definitive mandible, e.g., of the cartilaginous fishes.

The jaw bars rotate 180° from their position when the buccal cavity is wide open to their position when the buccal aperture

is tightly closed, and they carry the tentacles with them in this semicircular sweep. When fully expanded the tentacles form a widespread fringe encircling the buccal opening. When fully contracted the tentacles form an interdigitated and usually jumbled mass crowded into the buccal cavity and entirely filling it, and then the apparent buccal opening is formed by the contracted right and left metapleural folds.

There is thus evidence of abundant and varied muscular action, and the skeletal parts are so arranged as to give free and extensive change of motion of the parts. The jaw apparatus of *Amphioxus* has thus the motions of lateral biting jaws, but *Amphioxus* does not use them as such. It is important to note that the jaws can be and are frequently withdrawn inside the buccal cavity.

While the *Amphioxus* buccal skeleton is developed and adjusted to opening and closing the oral aperture, since the food of *Amphioxus* consists of minute organisms suspended in the respiratory current, no biting or comminuting function is needed. It is apparent how readily adaptable this apparatus is to such function, and when change of food habit occurred the jaws could be readily modified to meet the requirements of the new conditions.

Rising from the dorsal borders of the jaw bars, the right and left walls of the oral hood arch upwards to fuse together in the median line below the notochord; posteriorly they pass into the velar curtain which is perforated by the velar mouth (figs. 1, 3, 4, 8, 11, 12).

The tentacular rods are generally single, but branched rods occur in forked tentacles. The tentacles from the second quarter of the jaw bar reach to the tip of the buccal cavity when retracted and the short ones project outside (laterad) of them.

The tentacles are covered by extensions of the tissues of the jaw bar, consisting of a thick layer of surface epithelium and subepithelial connective tissue in which the nerves pass to their endings and the vascular channels ramify. The epithelium is produced at intervals into hillocks containing sensory cells.

The anatomy and histology of these structures is quite fully described in the literature of *Amphioxus*.

The two mesial tentacles at the base of the jaw bars differ from the others in being shorter and broader and they leave the

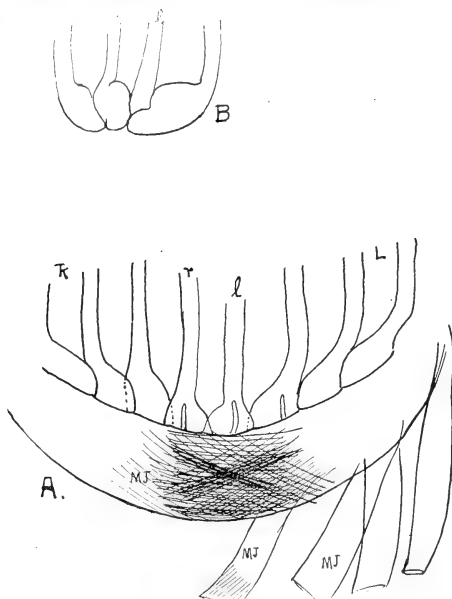


Fig. 2 Ventral view of the median part of the right and left jaw bars of *Amphioxus*. A shows the usual symmetrical arrangements of the tentacles on the bar. B shows an irregular arrangement; *r* and *l* are the first tentacular rods given off from the right and left jaw bars.

center of their basal plates, not at the anterior (distal) border as is the case with all the others. They have the appearance of palps.

The oral hood has the following attachments: 1) Directly continuous with the metapleural folds. 2) Strong tendinous attachment along the dorsal longitudinal lines of contact with

the mesial faces of the myotomes. 3) Bandlike tendons to the ventral edges of the myotomes. Dorsomesad of the dorsal longitudinal attachment to the myotomes there is a large lymph space separating the oral hood from the surface of the myotomes and

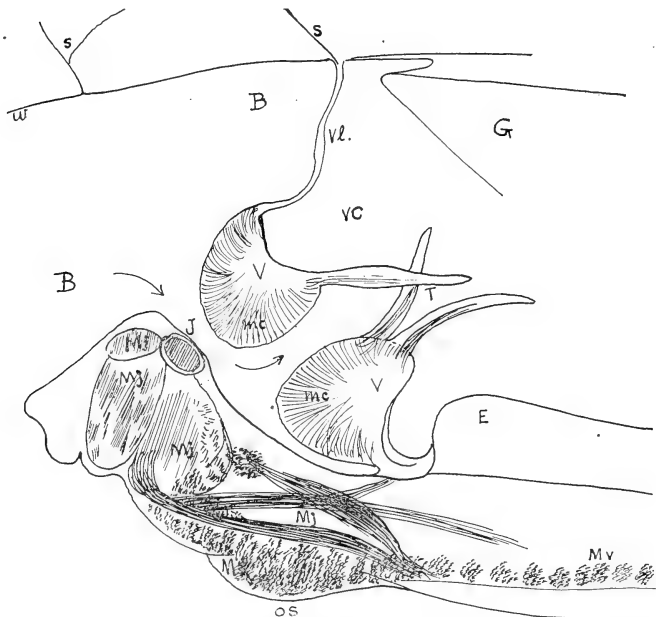


Fig. 3 Sagittal section of the head of *Amphioxus* near the median line to show the lower lip, jaw base, velum, and endostyle. The relations of the jaw muscles to the parent transverse muscle, to the velum, and to the jaw base are indicated. The jaw apparatus is in contraction.

the notochord across which connective-tissue threads connect to the adjacent structures.

The muscles which move the jaw bars and their tentacular projections appear to be derived from the large transverse ventral muscle of the body wall (figs. 2, 3, 4, 5, 6, and 7). They consist

of bundles of fibers turned in varying degrees out of the transverse direction of the fibers of the parent muscle bed. Thus all the jaw muscles may be said to have their origin from the ventral transverse muscle and to insert on the jaw bars and their

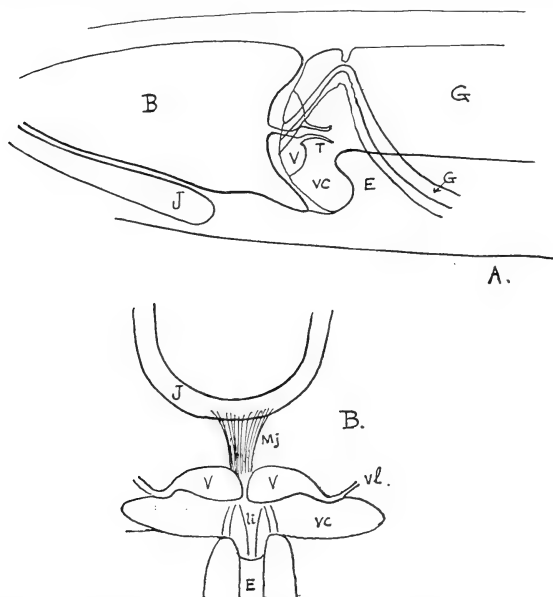


Fig. 4 A. Composite section to show relation of jaw base to velum of *Amphioxus* when jaw apparatus is expanded. The first three consecutive divisions of the gut are shown. B, buccal cavity; vc, velar chamber; G, pharyngeal canal.

B. Dorsal view of jaw base, velum, velar chamber, median part of gills, together with median muscle which arises from the transversus ventrad of the velum.

tentacular projections. The jaw muscles are divisible into two groups. The first group contains the muscles of attachment; the second group the intrinsic jaw muscles, yet the latter are in a way extensions of the former. In figure 3, the relations of some of these muscles are shown as they appear in median

section through the sagittal plane of the body. It will be noted how the ventral transverse muscle thickens as it approaches the base of the jaw bars, also how bundles of muscle fibers take on a longitudinal direction to insert on the jaw bars. Two large muscles and one small one which surround the base of the jaw follow the bars forward as the main intrinsic muscles of the jaw bars. The largest and most caudad of the three is seen to contain a large element of transverse fibers — the anterior bundles

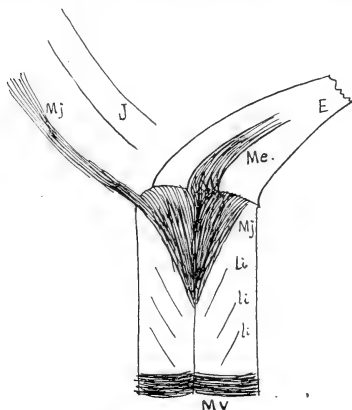


Fig. 5 Dorsal view of dissection of jaw base and ventral floor of velar chamber and endostyle of *Amphioxus*, showing the muscles attached to ventral surface of anterior end of endostyle arising out of the transversus and the lateral lines of attachment of endostyle to sheath of the transverse muscle.

of the transverse ventral muscle. One longitudinal bundle is shown which originates from the base of the endostyle and ventral border of the velum. In figure 2 is indicated the interdigitation and the crossing of these three bundles at the base of the jaw bars. In figure 4B and figure 5 is shown the bundle which arises from the base endostyle and velum.

In figure 6 the manner of origin of the left half of the jaw muscles from the transverse muscle to the left of the median line is indicated. Figure 7 shows a dissection of the jaw muscles,

their relation to the transverse ventral muscle, the jaw bars and basal tentacular palps.

The group of intrinsic jaw muscles has been previously described. It consists of a large fusiform bundle (outer muscle) running the entire length of the jaw bar of either side and a smaller muscle consisting of long bundles and short thin plates

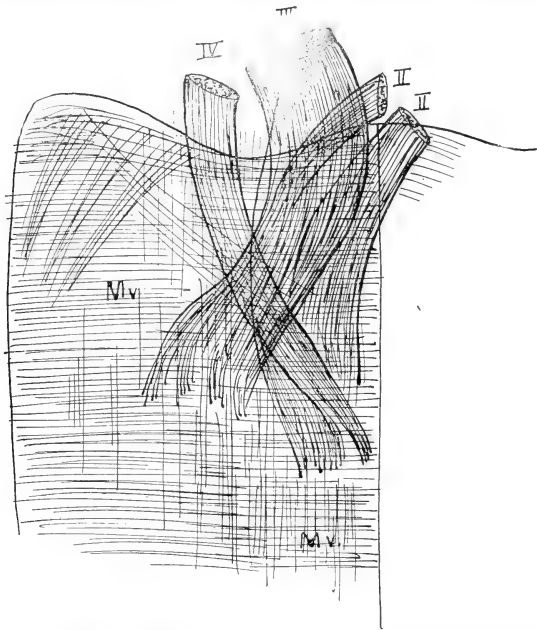


Fig. 6 Dorsal view of dissection of jaw muscles of the left side arising from transversus. *Amphioxus*.

of muscle fibers extending from one tentacular bar to the next one in front (inner muscle). These two groups we may call, one the mandibular muscle and the other the tentacular muscle. They are both complicated in their make-up.

The rectus-abdominis portions of myotomes 1 to 4, and sometimes myotome 5, are attached to the wall of the buccal cavity

along a horizontal line of insertion lying below the dotted outline of the dorsal border of the oral hood, as shown in figure 12. When the muscles of the jaw apparatus are fully contracted, the rectus abdominis section of each myotome contracts in unison to assist in withdrawing the jaw apparatus up into the boat-shaped cavity formed by the myotomes on each side and the notochord in

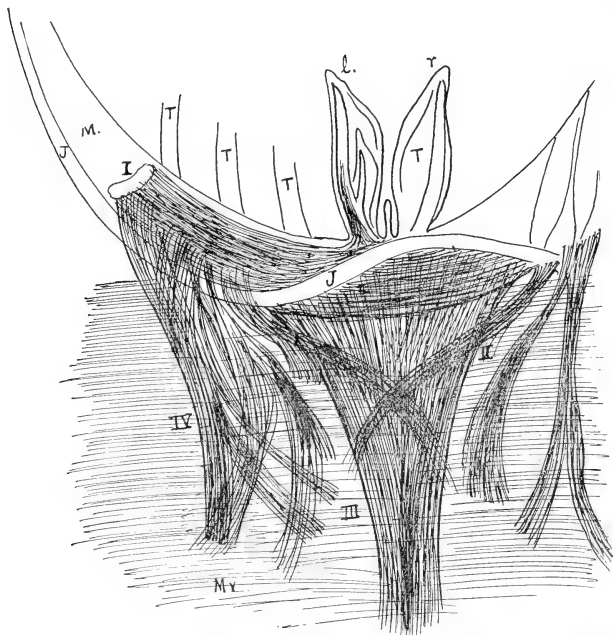


Fig. 7. Dorsal view of dissection of jaw base and its muscles. *Amphioxus*.

the middle and at the same time the metapleural folds are folded inward, the right over the left, so that the buccal opening is entirely closed and covered over and protected by these folds of the skin. The buccal cavity at such time is entirely filled by the tentacular projections of the jaw bars, crowded together like so many straws.

There are six pairs of muscles which move the jaw apparatus of *Amphioxus*. Based on their action they may be listed as follows:

I. Transversus abdominis, the parent muscle of them all. Is attached by tendons to basal part of jaw and supplies the bundles which compose muscles 2, 3, 4, 5. Assists in retracting the jaw apparatus and contracting the buccal cavity.

II. Retractor of the jaw apparatus, arises from transverse muscle and inserts into base of jaw. Draws the jaw apparatus backward and, in connection with the transversus, inward and upward into the buccal cavity.

III. Adductor of the jaw bars, arises from transverse muscle and inserts into basal part of jaw dorsolateral of the retractor. Rotates the mandible inward through an angle of 180° and adducts the jaw.

IV. Extensor of the jaw bars, arises from transverse muscle and inserts into basal part of jaw ventrolaterad of retractor. Rotates the mandible outward through an angle of 180° and extends the jaw.

V. Extensor of the jaw bars, arises from each segment of jaw and inserts into the more distal segments. The powerful extensor of the mandible.

VI. Adductor of the tentacles, arises from base of tentacles and inserts into next anterior tentacle.

All of these muscles, which have become largely longitudinal in direction, do not appear to have distinct sheaths; but from the clear-cut motions of opening and closing the buccal opening, retracting and in-folding the jaws and tentacles, it is certain that they have sharply defined mechanical pulls and are subject to an equally distinct nerve control. The whole jaw apparatus is anchored by tendons which extend from the muscles to the mesial surfaces of the body wall in front of the velum. Both tendons and slips of muscle extend from the velum to the jaw base.

The jaw apparatus as a whole is anchored by tendons to the ventral transverse muscle and to the tough aponeurosis covering the mesial surface of the myotomes. It functions in existing

Amphioxus: *a*) to keep the mouth, at times, wide open; *b*) to screen its entrance; *c*) to close it entirely; *d*) to serve on occasion as a strainer for the food current; *e*) to direct the food current to the oral aperture.

The major motions of the jaw apparatus are three. First, the motions of approximation and separation of the two arms of the loop, with the resultant interdigitation and separation of the tentacles. Second, the movement of elevation of the whole jaw apparatus to the roof of the oral hood so that its long axis lies nearly horizontal and parallel to the notochord, this movement affecting the posterior end of the loop most, and that of the depression of the jaw apparatus resulting in the increase of the vertical diameter of the buccal cavity. Third, the retraction and extension of the jaw apparatus due to the pull of muscles attached to the base of the loop and to the general contraction of the walls of the oral hood and body wall. The buccal framework can thus be withdrawn entirely within the oral hood or fully protruded as a projecting structure beyond the limits of the oral aperture.

The jaw apparatus arises in the young *Amphioxus* in the lip fold as two skeletal bars symmetrically placed on either side of the sagittal plane. These bars begin at the middle of the lower lip fold and grow outwards, then upwards, and forwards into and through the edge of the lip fold. The bars send out buds at intervals which later form the tentacles. These buds of the jaw bars push the muscular layer, the connective-tissue layers and the covering epithelium before them in such a manner that they fit over the skeletal axis of each tentacle as the finger of a glove covers a finger, except that the muscles extend only part way up the tentacle.

Amphioxus—jaw nerves

The researches of Hatschek, Heymans and van der Stricht, van Wijhi, Kutschin, Dogiel, and others, as well as my own, on the nerves of the head of *Amphioxus* have one outstanding result, viz., the oral hood is under the control of five pairs of

nerves from the central nerve cord which lie between the second nerve—the septalis—and the velum. These five nerves are the nerves of the jaw apparatus and velum, supplying also the walls of the buccal cavity and the other organs connected therewith, besides giving branches to the skin and myotomes. My own repeated studies of the head nerves of *Amphioxus* are in agreement with the major facts which may be classed as well established. There is great flexibility in the arrangement of the nerves of the head. The septal nerve and the eighth may also take part in the nerve supply of the jaw apparatus, but this is not usual.

The frequent presence of unusual dorsal roots and the occasional absence of one of the important visceral trunks are purely individual variations, and do not affect the large facts of the nerve supply of the jaw apparatus which are given here in condensed form for convenience of comparison with the nerve supply of the jaw apparatus of *Bdellostoma*.

An accurate drawing of the five nerves of the left side controlling the jaw apparatus, together with a posterior ventral branch of the septal nerve and the first postmandibular nerve or the eighth nerve counting from the terminalis, is shown in figure 12. The course of the visceral branches over the myotomes, and the larger branches to the jaw apparatus are accurately drawn. There is great variation in method of branching in different individuals, but this figure is typical for *Amphioxus lanceolatus*. The velar nerves are not drawn in, and only one of the deep branches turning mesad under the ventral edge of the body muscle is partly shown. In general these nerves show only a slight tendency to run cephalad, most noticeable where the ventral branches emerge from behind the myotome. Their general course is nearly due ventrad to their terminals, which in *Amphioxus* is the plexiform nerve net described by Fusari, Dogiel, and Kutchin as consisting of three main sections, an inner and outer jaw bar plexus and a more or less distinct tentacular plexus. From the plexiform structures the final terminal nerves are given off. The plexiform structures are associated with a longitudinal nerve trunk composed of a number of relatively large nerves running the

length of the jaw bar near the base of the tentacles made up of the larger nerve branches given off from the five jaw nerves. A satisfactory analysis of this longitudinal trunk, its components and the relations of the plexiform structures to it has not been

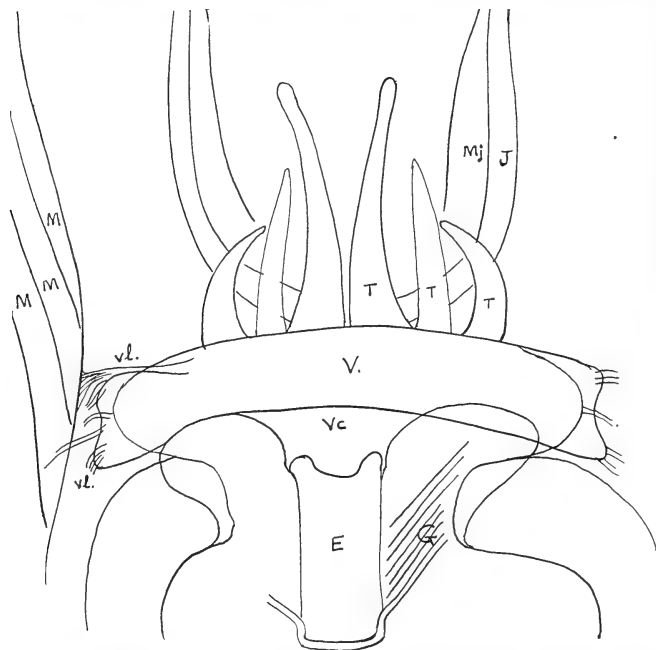


Fig. 8 Dorsal view of velum, velar tentacles and chamber, and anterior end of gill basket of *Amphioxus*. Six of the velar tentacles are protruded into the buccal cavity. The attachment of the velar ligaments is shown on the left side. The insertion of three myotomes is seen on the left side.

published. One or more branches of the longitudinal trunk run out into each tentacle. Thus the ventral branches of five pairs of segmental nerves of unmodified spinal type supply the jaw apparatus and exercise control of the whole mechanism

through a complex nerve-net system of fiber distribution which involves both sensory and motor terminals. The special asymmetry of the nerve supply confined almost entirely to the velum will be taken up later, but it may be said in passing

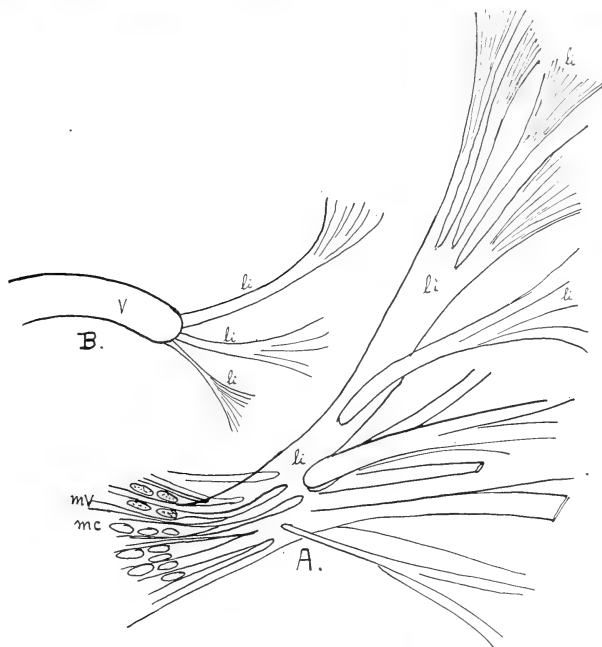


Fig. 9. Compound tendon connecting muscles of velum to body wall of *Amphioxus*. At the velar end, *Mc*, are shown a few cut ends of the circular muscles of the velum while the tendons pass into the radial fibers. At the distal end the tendon spreads out into a brush of fine tendinous threads which insert in the connective tissue wall of the myotomes.

that it does not affect the result of the innervation or the conclusions regarding the innervation of the jaw apparatus. Kutchin's observations that a branch of the third nerve of the right side furnishes a branch to the inner mouth plexus, hereto-

fore held to be formed by branches from left nerves only, shows that we have yet to learn the complete story of the innervation of the jaw apparatus. Even though van Wijhi's explanation of the asymmetry of the formation of the inner buccal plexus and the innervation of the velum is largely correct, the fact remains that the right side can and does supply its bilateral share to the buccal nerve supply of the inner plexus occasionally

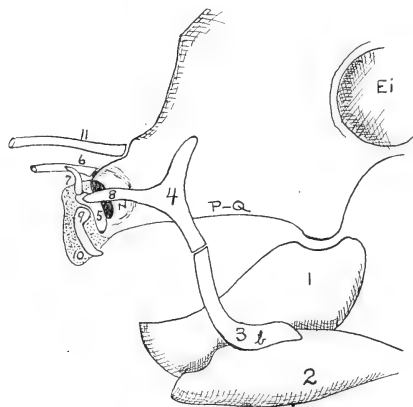


Fig. 10 Lateral view of left side of facial region of skull of *Callorhynchus* to show jaw apparatus. The remnant of the distal part of the jaw bar is attached to the hyoid as in *Bdellostoma* and runs forward and upward to the tentacular cartilages of the nasal region, thus preserving the ancient amphioxine jaw bar in a *Gnathostome* head.

if not frequently, and it would not require much more than has actually been observed to establish the complete bilateral symmetry in the descendants of *Amphioxus* which we observe in the higher forms, where the nerve supply of the right and left sides is usually finely balanced.

Thus the five nerve trunks innervating the jaw apparatus run ventrolaterad with a slight inclination cephalad. In the jaw their main branches run longitudinally. They leave the central nerve cord as separate and distinct dorsal roots. There

has thus been but slight displacement of the root portions of these nerves caudad. The septal nerve, while largely devoted to the sensory innervation of the snout, frequently sends one or two branches to the anterior end of the jaw apparatus.

The innervation of the jaw apparatus is quite sharply limited posteriorly. There is thus a section of the central nervous system

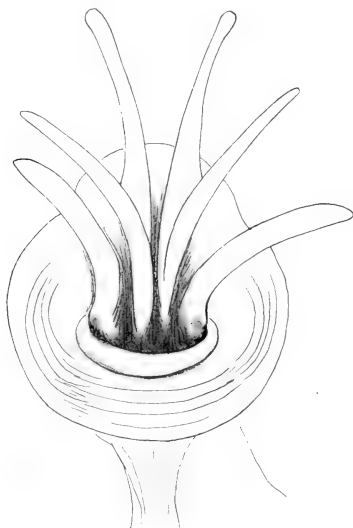


Fig. 11 Front view of velar disk of *Amphioxus* with the dorsal six of the velar tentacles projecting into the buccal cavity. When the lower six are thrust forward the lip fold disappears.

lying in front of the jaw apparatus whose peripheral nerves do not typically invade the jaw territory and another section of the central nervous system whose nerves supply territory normally behind the jaw apparatus; between these two lies a section of the central nerve cord which, to judge by the five nerves it sends out, is devoted exclusively to the control of the jaw apparatus. In the peripheral reaches of its nerves is built up a plexi-

form structure which does not appear to be duplicated in other peripheral territories.

The velum is a part of the jaw apparatus; it is the organ about which has centered all this structural activity. It is lodged between two folds of the mucosa which fuse to form the velar lip and the coverings of the tentacles, and is continuous with the lining of the buccal cavity anteriorly and the fold which is continuous with the lining of the branchial cavity posteriorly (figs. 1, 3, 4, 8). The velum of *Amphioxus* is a muscular dia-

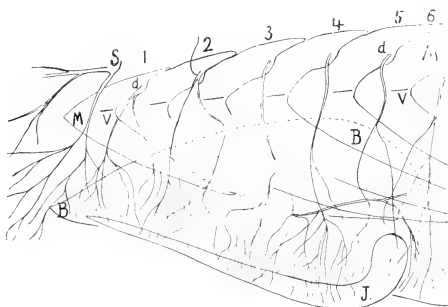


Fig. 12 Left side of jaw apparatus of *Amphioxus*. The dotted outline marks the position of the dorsal wall of oral hood. The relations of the first seven myotomes and the nerves 2 to 7 are accurately drawn. Nerves 1 to 6, the jaw nerves. The heavy dashes mark the position of the ventral spinal roots.

phragm. It varies much in shape, depending on the degree of contraction. When fully expanded it forms a thin circular ring with a large central hole, from the anterior rim of which twelve or more velar tentacles project forward into the cavity of the oral hood. In this state the radial muscles are contracted and the circular muscles are relaxed. This is the condition of the velum when the oral hood is wide open and the jaw apparatus fully expanded. When fully contracted the velum has the shape of a thick doughnut minus the hole and the tentacles project backward into the velar chamber or are folded across the posterior face of the muscular lump. When *Amphioxus* is killed with

quick-acting reagents (e.g., osmic acid) it often happens that the velar tentacles are not all withdrawn and some of them still project forward (fig. 11), while others project backward. In life the velar tentacles were long ago observed to occasionally flip back and forth while the velum was fully expanded. Their

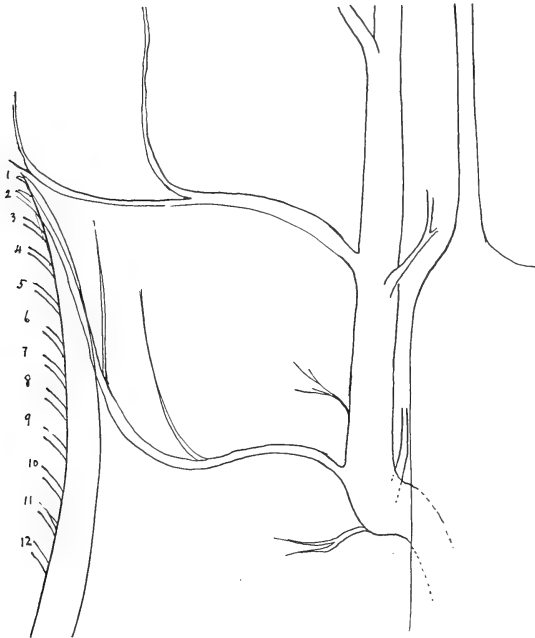


Fig. 13 Part of the brain and the terminal and septal nerves of *Amphioxus*. Two branches of the latter supplying the anterior end of the jaw apparatus are shown.

normal position while the food current is flowing in through the velar aperture is that of full erection projecting forward into the oral hood, forming a funnel-shaped fence about the velar mouth. In the normal closure of the velum the tentacles are folded toward the center and caudad due to the fact that the

radial muscles of the posterior face of the velum insert into the base of the tentacles and draw the lip caudad, thereby flexing the tentacles caudad and pulling the lip caudad of the muscular diaphragm. In expanding, the motions are the reverse of these. The velum is firmly tied to the surface of the myotomes on each side as well as to the base of the jaw apparatus in front and the gill basket behind. It thus forms a flexible curtain between the buccal cavity and the gill chamber.

AMMOCOETES

The jaw apparatus of *Ammocoetes* is laid down after the same plan as that of *Amphioxus*. It consists of two lateral jaw bars of soft cartilage and procartilage, which are largest at their union in the midventral line and taper to their anterior dorsal ends located on the ventral surface of the upper lip. They form the boundary of the buccal aperture with their proximal vertical portions and inclose between them a remnant of the ancestral buccal aperture of *Amphioxus* in their horizontal distal portions. They bear tentacles throughout their entire length (figs. 14, 15, 18, 19, and 21).

In the midventral line they fuse, and from this junction is given off dorsally a flask-shaped body which is crowned by the median tentacle, and ventrally the jaw bars are continued caudad in the form of a large spike of cartilage or median projection of the jaw bars (figs. 15, 18, 19, 21).

A reduction of the distal part of the jaw bars together with an extension of their proximal portions has taken place since the *Amphioxus* stage was passed, but the amphioxine character of the jaw bars is plain to see. Viewed from in front the jaw bars curve outward and upward nearly vertically from the midventral line, then inward, but do not meet in the middorsal line, thus forming an incomplete ring with the opening above. Up to this point they are inclosed in the lips of the buccal aperture. The ends of the open ring lie at the junction of the upper lip with the head. Here the jaw bars bend forward nearly at right angles and lie on the ventral surface of the upper lip, and the position of the anterior part of the ancestral buccal chamber

is marked by the tentacle-covered surface which leads back into the buccal opening between the vertical parts of the jaw bars. Thus the posterior part only of the ancestral buccal aperture is functional in *Ammocoetes* (figs. 14, 18).

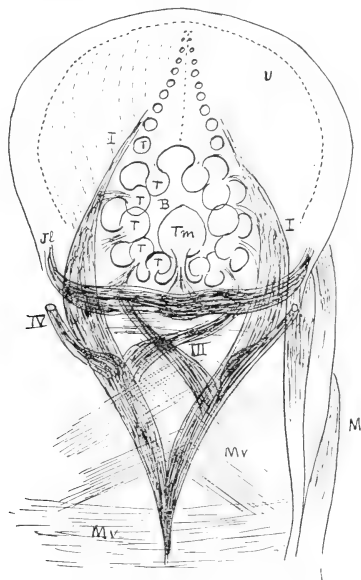


Fig. 14 Upper lip of a 15-cm. *Ammocoetes* seen from ventral surface. The positions of jaw bars are in part outlined by two of the rows of tentacles and the long muscle of the jaw between the rows of tentacles. In front of the functional buccal aperture is included the remains of the anterior part of the buccal aperture of the amphioxine ancestor. The number of rows of tentacles and consequently the width of this space varies in different stages of growth. The jaw muscles shown are those belonging to the jaw bars and tentacles and the muscles at the base of the jaw apparatus. On the right side of the figure the forward extension of the parietal muscles is shown. They attach to the upper and lower lips. In front of the buccal aperture only the two main rows of tentacles are indicated. In this 15-cm. *Ammocoetes* there are four additional rows of smaller tentacles on each side. The dotted outlines show the boundary of the tentacular region in an 8-cm. *Ammocoetes* and the rows of dashes the position of rows of small tentacles of this stage. The median line of dots marks the position of a median line of small tentacles sometimes present.

When seen from the side (fig. 18), the proximal part of the jaw bar stands perpendicular to the ventral bar. Where the upper lip joins the head the jaw bars bend from the vertical to a horizontal position and run forward in the ventral part of the upper lip just below the skin, converging as they go forward.

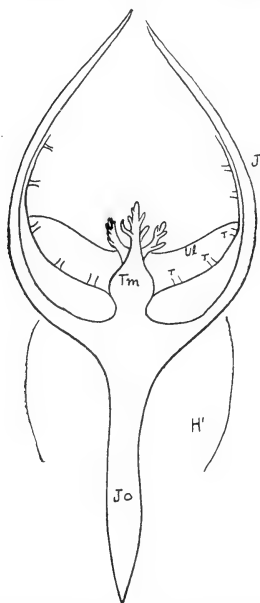


Fig. 15 Dorsal view of the skeleton of the jaw apparatus of Ammocoetes, showing the jaw bars, bases of part of the tentacles, the large median tentacle, the extent of the hyoidean skeleton and outline of the lower lip.

The vertical portions of the jaw bars form the elastic skeletal support of the buccal aperture. The basal median bar is a more or less cylindrical piece with its largest diameter where it unites with the jaw bars; here it gives off a short process which projects forward and upward, swelling into a bulb or flask-shaped body with a slender neck crowned by a bush of tentacular branches

which are mainly directed forward and upward. When the tentacle is fully erected the branches stand nearly vertical to the long axis of the body. About this bush are grouped ten other similar bushes, the crowns of ten lateral tentacles, five on each side. These bushes are so set that their branches interdigitate, forming a screen which separates the functional part of the buccal cavity from the outside (figs. 14, 18).

The posterior part of the median bar projects caudad in the ventral wall of the body as far back as the branchial region, where it tapers to a point in the connective-tissue framework of the body wall. Below the base of the jaw bars and the median bar is a sheet of procartilage which thins out laterally as it passes upward in the body wall on each side and also as it runs caudad below the median bar. This sheet of procartilage gives rise to the hyoidean apparatus of *Petromyzon* (figs. 15, 21).

As in *Amphioxus*, the jaw bars bear tentacles their entire length. Present as a single row about the functional part (buccal aperture) they appear in a varying number of rows on the surface of the lip, the rows being more numerous in younger than in the older stages of growth. The tentacles vary from short finger-shaped projections, such as are given off at the distal extremity of the rows, through all gradations to the bushy structures given off from the base of the jaw bars (fig. 16). The branching is dichotomous. As Prof. S. H. Gage has explained, this group of tentacles forms a strainer or sieve in front of the buccal cavity. Along the horizontal part of the jaw bars the tentacles project from the ventral concave face of the upper lip, due to the spreading out of the skin cover of the jaw bars as part of the lip covering. They decrease in size toward the tip of the bars and also from the mesial rows laterad. They form converging rows of fungoid outgrowths, coursing laterad, the more posterior ones terminating in the border of the tentacular area, the anterior (mesial) ones converging toward the middle line near the front border of the lip (fig. 14). The median tentacle mentioned above is the largest of all and surmounts a bulbous structure which after the metamorphosis appears as part of the skeleton of the mandibular mechanism of *Petromy-*

zon. Kaensche has described the part taken by the skeleton of the median tentacle in forming the jaw of *Petromyzon* as well as the origin of the hyoid from the sheet of procartilage of the *Ammocoetes* which lies ventrad of the jaw bars. The buccal screen is figured by Professor Gage on plate VI, figure 22, and, as he states, this tentacular sieve prevents the entrance

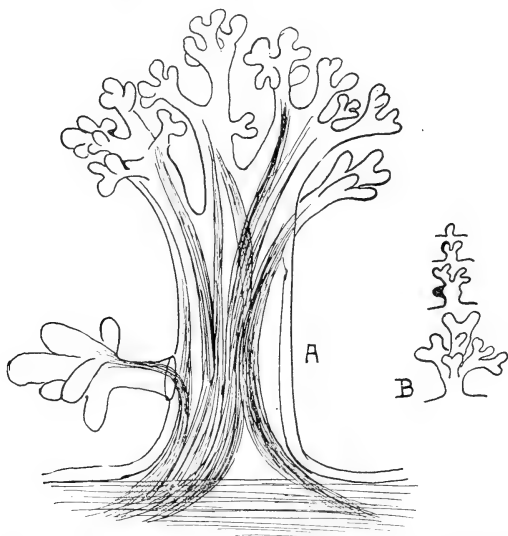


Fig. 16 A. One of the larger-branched tentacles from the jaw bar.
B. Four small tentacles to show method of branching.

of large particles into the buccal chamber and when tightly closed shuts off the flow of water through the buccal aperture.

The mandibular bars are not so conspicuous superficially as in *Amphioxus*, but they occupy the same relative position in the head. Due to the shortening of the notochord, the forward overgrowth of the brain, and the housing of the nasal organs, the head does not project beyond the boundary of the buccal chamber marked out by the proximal part of the jaw

bars. The head has also acquired a large muscular upper lip which carries on its undersurface the anterior part of the territory of the oral hood, or correctly put, the distal part of the oral hood has acquired a muscular roof which has suppressed the cavity. The muscles of the mandibular bars consist of two tapering fusiform bundles of fibers which accompany the bars, flattening and thinning out on the horizontal part of the jaw bars.

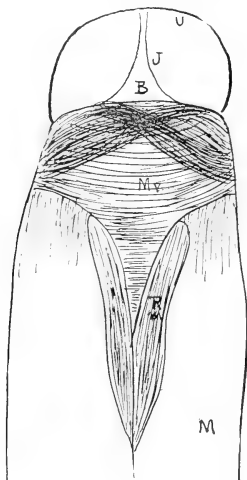


Fig. 17 Ventral view of superficial muscles of 10-cm. Ammocoetes, to show the transverse muscle and its derivations that lie in the lower lip.

At the base of the jaw bars they are thicker than the bars and arise from the lateral borders of the median bar and the floor of the buccal chamber. They pass forward, outward, and upward along the outer border of each jaw bar. Where they originate they interdigitate with the circular muscles of the head. They furnish the tentacles with muscles, sending off shoots into the larger ones (fig. 16), which attach to the walls. The jaw muscle is attached to the skeletal tissue at the base of all tentacles, both large and small. The motions of the jaw bars in Ammocoetes are not as varied

or extensive as in *Amphioxus*, owing to the fixation of the anterior part of the jaw apparatus to the upper lip. The contraction of the vertical portions of the jaw muscles closes the buccal aperture and they are aided in this action by the circular muscles of the head, which form a special sphincter surrounding the buccal aperture outside the jaws. The expansion of the buccal aperture seems to be largely due to the elasticity of the chondral jaw bars aided by the ventrolateral retractors of the jaw bars.

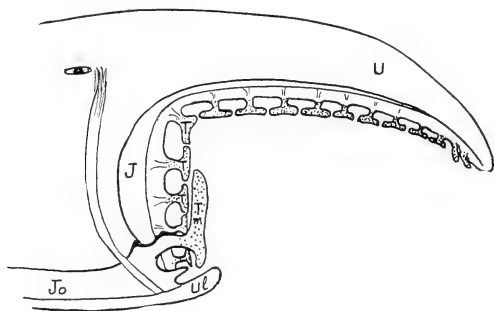


Fig. 18 Composite picture of sagittal sections of head of *Ammocoetes*. The position of the eye is indicated, as well as the circular muscle which begins in front of the eye and runs ventrad to insert on the jaw apparatus. The left jaw bar with its tentacles and the median tentacle are shown as though lying in the plane of the section. The tentacles are outlined as though solid bodies.

The head muscles of the *Ammocoetes* consist of the parietal or trunk muscle, the branchial, velar, buccal, jaw, and lip muscles. The buccal and velar muscles are composed of two groups, the ring and the longitudinal. The ring or transverse muscles consist of two layers of fibers separated by a layer of procartilage. They are the continuation forward of the muscles of the branchial basket. The group of buccal muscles attach to the base of the cranial skeleton, both the cartilaginous and membranous cranium, and to the nasal capsule. The general direction of the fibers is ventrad in the sidewalls of the head, to curve inward from right to left to the fusion of the muscles of the two sides along the median ven-

tral line; the only indication of a median raphé is the interdigitation of some of the muscles, those not interdigitating have their fibers continuous. In front of the nasal capsule the dorsal

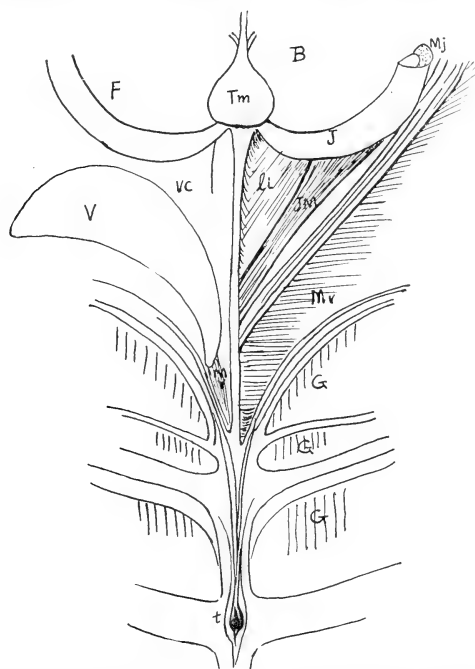


Fig. 19 Dorsal view of the floor of the buccal cavity, velar chamber, and anterior end of branchial cavity of *Ammocoetes*. The right and left arms of the jaw bar are shown to the left and right of the median tentacle, also its median tendon extending caudad in the ridge in which the opening of the thyroid gland occurs. The velum with the ventral attachment of its muscle and the position of the anterior gills are also shown.

layer runs into the upper lip and the bundles cross each other. Below the capsule two heavy bundles arising from the lateral surface of the ring muscle next the mucosa run into the upper lip, crossing one another in front of the palatine loop. In the

ventral body wall, especially in the velar region, the fibers of the ring muscle interlace across the middle line of the body.

The velar muscles arise from three territories: first, a strong group from the skull behind the eye; second, a band of fibers, not continuous, from the lateral part of the neighboring ring muscle; and, third, a long but heavy bundle from near the mid-

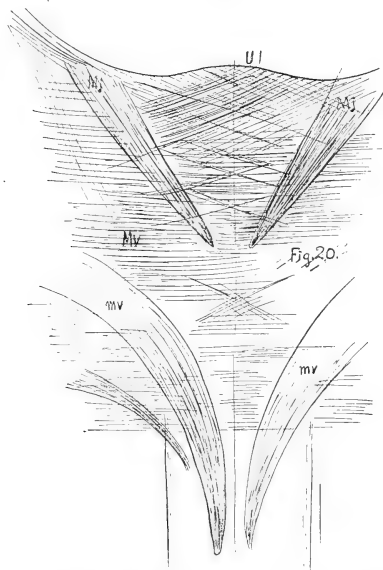


Fig. 20 Ventral view of the jaw and velar muscles, derivatives of the transverse or circular muscles of the head. *Ammocoetes*.

ventral line of the branchial skeleton. This latter bundle is long, and sweeps forward, upward, and outward into the velar body to form the most important muscle of the organ. It is the retractor veli. The velum is greatly assisted in closing by the elastic velar cartilage.

Besides the large jaw muscle already described, which lies on the outer face of each jaw bar and spreads out on the ventral

face of the upper lip, other muscles belonging to the constrictor group assist in the control of the jaw apparatus through their insertion into the base of the upper lip. The upper lip muscles as a whole form a concavoconvex muscular flap attached to the anterior end of the head—indeed, the upper lip is the muscular roof of the oral-hood territory and the jaw bars and tentacular skeleton form its main support. Muscle bundles run into it from the right and left sides from above and below and the lower lip is a unit part as far as the muscles are concerned. The

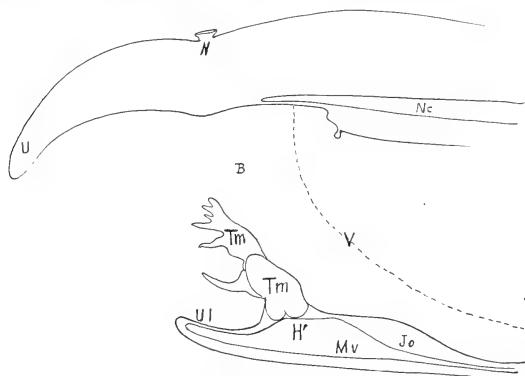


Fig. 21 Sagittal section of the anterior end of the head of Ammocoetes, to show the jaw bar in the median plane with the large tentacle, the position of the velum and the dorsal body wall.

lips thus form a funnel with the lower third of its wall cut away almost back to the jaw bars.

The jaw apparatus is under the control of the trigeminus and facialis. I have not given these nerves of Ammocoetes the necessary analytical dissection, and cannot therefore make comparison with the nerves of the jaw apparatus in Amphioxus. The Marsipobranch innervation will be described as it occurs in Bdellostoma. Here a thorough dissection shows the Marsipobranch innervation of the jaw apparatus is fundamentally in harmony with that of Amphioxus.

BDELLOSTOMA

Great as the structural differences between *Amphioxus* and *Ammocoetes* undoubtedly are, they are not in some respects greater than the structural differences between *Ammocoetes* and *Bdellostoma*.

It is an accepted fact that *Ammocoetes* has progressed much further toward the condition of anatomical organization found in fishes higher in the scale of development than has *Amphioxus*. It is also true that *Bdellostoma* has reached a much higher state of organization than *Ammocoetes* and in general in the direction toward fishes reckoned as more advanced in structure, e.g., the Chimaeroids and sharks. It is generally held that the Myxinioids are not jaw-bearing fishes, although the evidence is accumulating to prove that they are. Having presented evidence to show that the buccal skeleton and some associated structures in *Amphioxus* are homologous with the parts of the jaw apparatus of *Ammocoetes*, if we can now show that the heretofore puzzling jaw apparatus of *Bdellostoma* is built on the same plan and can establish the essential homology of its parts with these of *Amphioxus* and *Ammocoetes*, we shall advance far in our effort to solve the jaw problem.

The skeleton, muscles, and nerves of *Bdellostoma* have been subjected to thorough investigation, but have not by any means yielded up all they have to tell. No complete description of these structures will be given here, but only such details as seem pertinent to the problem under consideration.

The skeleton of *Bdellostoma* is both cartilaginous and membranous; many structures important for comparison with higher forms are still in the membranous stage of their development. The comparison of the parts which have become cartilaginous with the same membranous territories in *Ammocoetes* and *Amphioxus* is indeed instructive and the same may be said for cartilaginous parts in the higher fishes which remain membranous in *Bdellostoma*. In such comparison is a rich mine of morphological data.

For more detailed accounts of the anatomy of the skeleton, muscles, and nerves the reader should consult the works of

J. Müller covering a general survey of the anatomy of *Bdellostoma*, Ayers and Jackson on the anatomy of the skeleton, Julia Worthington on the head nerves, E. P. Allis on the skeleton, nerves, and muscles of the head, F. J. Cole on the skeleton and muscles of *Myxine*, and P. Fürbringer on the skeleton, nerves, and muscles of *Myxine*.

The jaw apparatus of *Bdellostoma* consists of a pair of dentigerous jaw plates located in the buccal cavity and lying above a large cartilaginous plate which forms a support for it, of a pair of bars starting from near the sides of the jaw plates, but

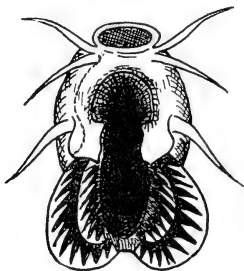


Fig. 22 Front view of face of adult *Bdellostoma*, with jaws protruded, showing external relation of tentacles to narial and buccal apertures. With the hyoid as a base, the jaw bars pass upward giving off the tentacles and framing in the buccal opening. The dotted line on the left side indicates the course of the distal part of the jaw bars in front view.

attached to the large support plate which lies ventrad of the jaw, and which run forward and upward in the lips of the buccal cavity (figs. 22, 23, 24, 25, 26, 27, 29). They attach at their distal ends to the lateral borders of the nasal tube near to its anterior end. The first part is the basal or proximal part of the Amphioxine jaw bar and the second part is the distal section.

The proximal part has been transformed into a tooth-bearing protrusible jaw, while the distal part serves as the skeleton of the lips of the buccal aperture and is the only part bearing tentacles in *Bdellostoma*. The number of tentacles is reduced to four, two of which (the first and third) are unit structures

with the cartilaginous jaw bar, while the other two (the second and fourth) are connected only by tendons to the jaw bar. The cartilages of the tentacle, two on either side, are fused together where they meet in the middle line below the nasal tube, and through this bar they are connected with the subnasal cartilage. The cartilages of tentacle four lie in the heavy lip folds at the sides of the ventral part of the mouth opening, and are so held by tendons and muscles as to serve as a firm support for the pair of thick flaps of skin which operate as a mouth guard and jaw cleaner, as well as a palp. The other three tentacles are sensi-

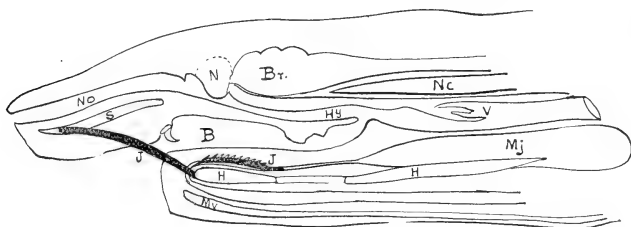


Fig. 23 Composite figure from sagittal sections of head of adult *Bdellostoma* showing outlines of important parts of jaw apparatus. The tentacular part of the jaw bar, of course, lies in the side of the head. The tooth-bearing jaw cartilage is also shown, but it also lies at one side of the median plane.

tive fingers used in connection with both nasal and buccal apertures.

The important relation is that the jaw bars are the skeletal framework of the buccal aperture just as they are in *Amphioxus* and *Ammocoetes*. We found in *Ammocoetes* that a new skeletal structure had been added ventrad of the base of the jaw bars and that this sheet of procartilage of *Ammocoetes* developed into the submandibular cartilage of *Petromyzon*. In *Bdellostoma* we find this element larger and assuming proportions unusual, so much so that Prof. G. B. Howes called it "the dominant monster of the Hag." It is however, admirably adapted to its functions. It serves as a traveling floor for the jaws in their extensive excursions out of and into the buccal cavity. Its

front edge also serves as the fulcrum over which the powerful pulls are exerted in the rasping and tearing action of the teeth of the jaw. It further serves as the firm base of attachment of a large group of muscles which take part in the work of operating the jaw and in closing the buccal cavity as a whole.

The attachment of the distal ends of the jaw bars to the anterior end of the base plate or hyoidean apparatus is well accounted for by the close association of the two structures both in position and in functioning and by the necessity for the separation of the distal from the proximal division of the jaw bar in order to secure freedom of motion of the latter. The position of the distal part of the jaw bar varies with the degree of contrac-

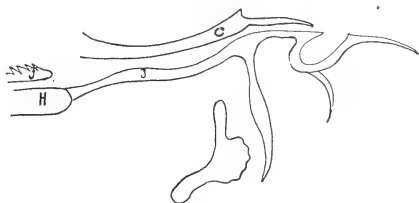


Fig. 24 The adult cartilaginous skeleton of the jaw apparatus of the right side, including the corneal cartilage, anterior end of jaw and hyoid to which the jaw bar attaches. *Bdellostoma*.

tion of the snout region of the fish, but, as figures 22, 23, 24, 25, 26, 29 show, it runs forward and upward at an angle that recalls the slope of the jaw bars in *Amphioxus*.

The hyoidean apparatus has acquired cartilaginous connection with the skull above (figs. 25, 26, 27), and is also connected thereto by strong tendinous bands. The jaw itself has no attachment to the cranial skeleton except the tendinous attachment to the anterior end of the nasal capsule and a similar connection through the corneal cartilage with the palatine and subnasal bars. As the jaw works longitudinally while in the head, it has no vertical stresses not already provided for by the hyoidean supporting mechanism, and it is the latter which has developed the cranial attachments mentioned above.

The muscles of *Bdellostoma* have been differentiated to such a degree beyond the *Ammocoete* condition that they have not yet been reduced to a grouping that permits comparison with *Ammocoetes*. I had planned to publish an account of the muscles of *Bdellostoma* in 1898; it would not have cleared many of the problems seeking solution. That paper went to the wastebasket two years later. Since then I have sought a basis for comparison of the muscles of *Bdellostoma* with both lower and higher forms. In 1896, F. J. Cole published a very full account

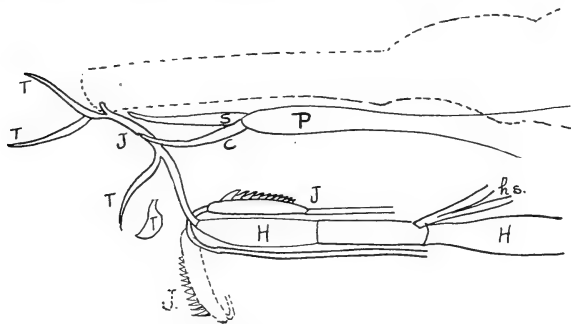


Fig. 25 Dissection of the left side of skeleton of jaw apparatus of *Bdellostoma*, to show the relation of its parts with dentigerous jaw protruded and retracted. The hyoidean mechanism bears the tentacular portion of the jaw bars fastened to its anterior end, thus leaving the dentigerous jaws free to move unhampered.

of the muscles of *Myxine* with frequent references to the muscles of *Bdellostoma*, but he recognized that the comparative morphology of his subject was not ripe for solution. What is here offered is a grouping of the Myxinoid muscles based on the comparative anatomy of the jaw apparatus as outlined within.

It is evident that we have to deal with two quite distinct major groups of muscles associated with the jaw apparatus. The first and oldest group is that of the intrinsic muscles of the jaw apparatus which are developed out of the ventral plate of transverse muscle fibers of the *Amphioxine* ancestor. Already in *Amphioxus* the group of intrinsic muscles is sharply

defined, and they only grade into the ventral transverse muscles where the bases of the jaw bars attach to the parent muscle. In *Ammocoetes* we have the system of constrictors quite complete as such, forming a broad girdle of muscle bands encircling the branchial pharyngeal and velar region, attaching to the axial skeleton above and holding the base of the jaw bars in their grasp. With many interruptions of the continuity of the ring muscles in the branchial region these groups of muscular girdles send out offshoots—to the velum, to the jaw apparatus, and to the lip flaps. The phylogenetic history of these ring-

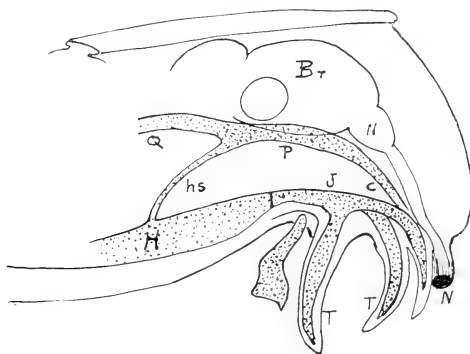


Fig. 26 Side view of dissection of head of 37-mm. *Bdellostoma* embryo, to show relation of jaw apparatus at this stage. The dentigerous jaw is omitted. The abrupt bend ventrad of the nasal region is shown, as also the folding of the skin on the dorsum of head.

muscles is not clear. We find in *Ammocoetes* the hyoidean apparatus laid down in procartilage within the ring-muscle system and extending from below the jaw bars backward. It cares for stresses beyond the function of the muscles, but the ring-muscle bundles still largely retain their continuity as rings. In *Bdellostoma*, however, a larger and more active fish, the ring-muscles have been broken into segments of rings by the very great development of the hyoidean apparatus in both cartilaginous and membranous form and the segments of the ring muscle of the *Ammocoetes* stage now insert, by muscle ends

on the cartilages of the hyoidean apparatus and the cranial skeleton which has also developed a number of new cartilages and increased the relative size and strength of its tendon and fibrous bands. The muscles of *Bdellostoma* may be grouped according to derivation as follows:

Group 1. Muscles of the jaw apparatus. The most ancient muscles of the vertebrate head, not considering the but little differentiated myotomes. The muscles of this group are all derived from the ventral transverse muscle of *Amphioxus*. Some of them have already acquired a longitudinal direction in *Amphioxus*. This process is continued in *Ammocoetes* and *Bdellostoma*.

A. Muscles of the jaw bars

a. Of the dentigerous jaw:

Retractors

- | | |
|---------|--|
| Md..... | 1. Mandibularis (longitudinalis linguae) |
| Md..... | 2. Perpendicularis |

Protractors

- | | |
|--|---------------------------------|
| | 3. Hyo-copulo-glossus |
| | 4. Copulo-glossus superficialis |
| | 5. Copulo-glossus profundus |

b. Of the distal part of the jaw bar and tentacles:

- | | |
|-----------|------------------------------------|
| Oph..... | 6. Nasalis |
| Oph..... | 7. Tentaculo-posterior |
| Oph..... | 8. Tentaculo-ethmoidalis |
| Oph..... | 9. Transversus oris |
| Oph..... | 10. Ethmoideo-nasalis |
| Oph..... | 11. Palato-ethmoideo-superficialis |
| Max?..... | 12. Palato-ethmoideo-profundus |

B. Muscles of the hyoidean apparatus

- | | |
|-----------------|------------------------------------|
| Max..... | 13. Palato-coronarius |
| Max..... | 14. Copulo-tentaculo-coronarius |
| Max?..... | 15. Coronarius |
| Max..... | 16. Copulo-ethmoidalis |
| Md..... | 17. Copulo-copularis |
| Max (VII?)..... | 18. Copulo-palatinus |
| VII..... | 19. Hyo-copulo-palatinus |
| VII..... | 20. Copulo-quadratus superficialis |
| VII..... | 21. Copulo-quadratus profundus |
| VII..... | 22. Cranio-hyoidens |
| Max..... | 23. Quadrato-palatinus |

C. Muscles of the velum

- | | |
|----------|--------------------|
| Md..... | 24. Velo-quadratus |
| Md?..... | 25. Velo-spinalis |

D. Muscles of the pharynx and gills

- IX..... 26. Constrictor pharyngis
 IX..... 27. Constrictor branchiarum et cardiae

E. Muscles of the trunk

- sp..... 28. Parietalis
 sp..... 29. Obliquus
 sp..... 30. Rectus

F. Muscles of the cloacal region

- sp..... 31. Sphincter cloacae
 sp..... 32. Transversus caudalis
 sp..... 33. Cordis caudalis

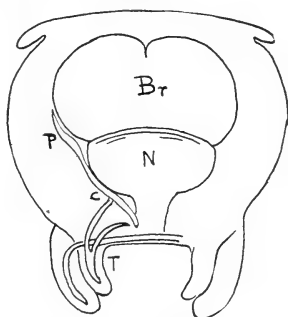


Fig. 27 Front view of the same dissection (37-mm. embryo), to show relation of the external tentacles to mouth and the cartilaginous skeleton of the jaw apparatus.

This may be subject to some changes as a result of further study of the interrelations of the muscles and skeleton, particularly after a study of their embryology and a more complete knowledge of their innervation which as published is both inaccurate and incomplete.

The attachment of the mandibular mechanism to the base of the skull and its association with the maxillary mechanism is a remarkable work of coördination and correlation which has taken place in the vertebrate stock above the Myxinoids. An interesting variety of experiments involving the use of numerous mechanical devices is presented by the cartilaginous fishes,

not to mention other groups. The acquisition of biting jaws was an important event in the evolution of vertebrates, for it marked a great advance in the mechanics of food-gathering and of defence. What advance, if any, *Bdellostoma* has made toward the acquisition of a maxillary mechanism is not particularly clear. Allis is the only investigator who has sought to identify the maxillary element by comparative means, taking into consideration skeleton, nerves, and muscles. His conclusion that the cartilage of the fourth tentacle represents the cartilaginous

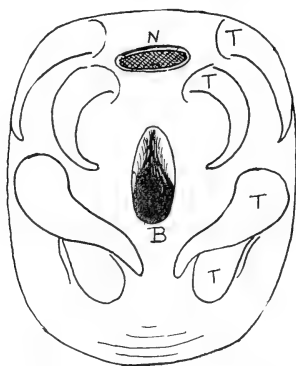


Fig. 28 Face view of 37-mm. embryo before dissection was made for figures 26 and 27. Note the small buccal aperture and the relations of tentacles to both nasal and buccal openings.

precursor of the osseous maxilla of Ganoids and Teleosts and that the lateral labial cartilage is in fact the cartilaginous palatine element does not appear to me to be well founded. I am not yet willing to say that it is impossible, but it is very improbable in my judgment. This much is certain, the fourth tentacle is used by *Bdellostoma* as an antagonist to the jaw as already described, in that it is at times pressed against the dental surface of the dentigerous mandible. In the event the fourth tentacle proves to be the nascent maxilla, we would have the whole mandibulo-maxillary apparatus of vertebrates developed out of the Amphi-

oxine jaw bars, which would be a peculiarly fit use to make of such building material.

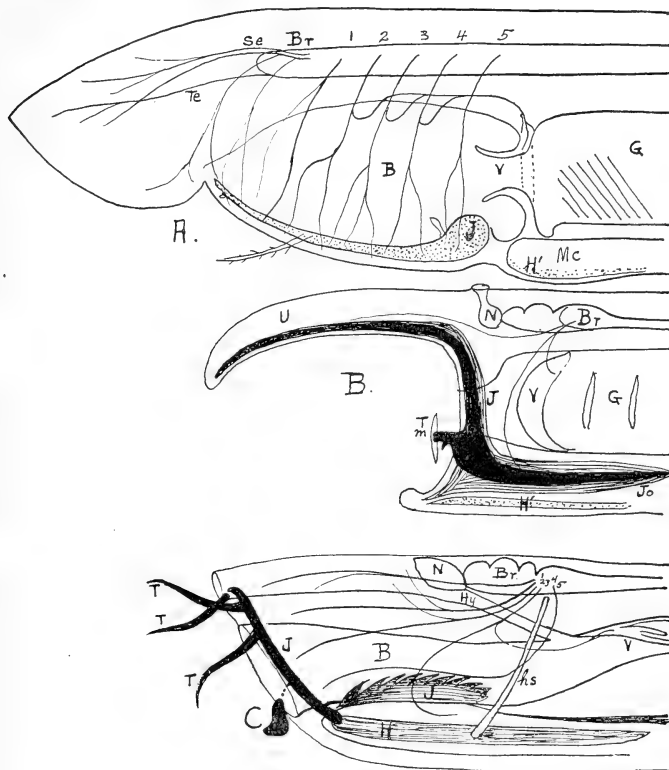


Fig. 29 Three figures to show the basic similarity in the arrangement of the jaw apparatus in A. *Amphioxus*, B. *Ammocoetes*, C. *Bdellostoma*. 1 to 5 are five nerves of jaw apparatus.

When the jaw is protruded the fourth tentacle lies above and upon the exposed dental surface, and when the jaw is being retracted the tentacular flap serves to clean the edge of the jaw of foreign particles.

Amphioxus, Ammocoetes, and Bdellostoma, in the order named, represent three stages in the phylogenetic development of vertebrates. They are the only known living representatives of the earliest vertebrate forms, as far as our knowledge reaches.

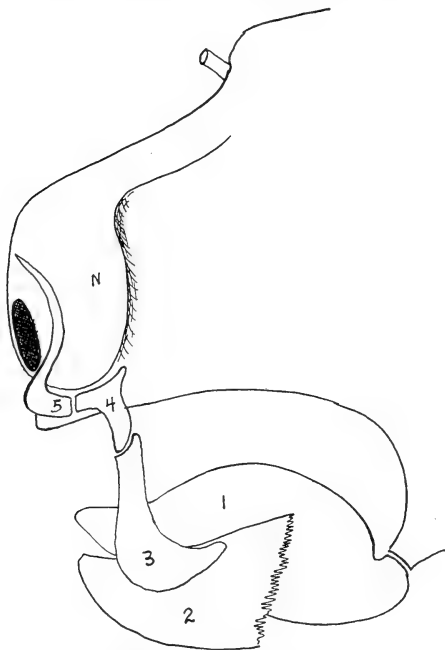


Fig. 30 Left side of facial region of skull of Chimaera, to show amphioxine jaw apparatus still in existence in a Gnathostome. 1 to 9 are parts of jaw bar and tentacles.

In view of the great structural differences between Bdellostoma and the Chimaeroids and sharks, the extensive changes involved in the evolution of the Amphioxine type into the Myxinoid type and our incomplete knowledge of both the anatomy and embryology of the latter it is evident that additional studies are needed if we expect to arrive at satisfactory solutions of the

problems concerning the phylogeny of the organs of the vertebrate body.

The solution here offered of the Trigeminal complex of *Bdellostoma* is the outline of a new description of these nerves based on my investigation of the head nerves of this fish. Out of the several hundred dissections made in detail, to cover the relations of each nerve as completely as possible, finally came the realization that the trigeminal complex of *Bdellostoma* is arranged after the *Amphioxine* plan and apparently inherited directly from *Amphioxine* ancestors. Although the relations of the five segmental nerves which compose the nerve supply of the *Bdellostoma* jaw apparatus are masked by being crowded together and bound up in dense connective sheaths, they can be made visible, and their homology then becomes evident. The figures 31 to 35 are drawn from dissections and are selected from a large number made to illustrate the anatomy of these nerves. They show some details of root origins and terminal distribution of importance in establishing the structural similarity of the innervation of the jaw apparatus in *Amphioxus* and *Bdellostoma*. It is not intended to fully describe these nerves, but merely to present certain features pertinent to their comparative anatomy as jaw nerves.

A detailed account of the head nerves of *Bdellostoma* will be given in another contribution. Miss Worthington, Allis, and Müller have described the head nerves in *Bdellostoma* and Fürbringer and Bridge those of *Myxine*, to mention the more important contributions. The ophthalmic nerve is uniformly called the first or anterior nerve of the group.

In order to lay bare the medullary bundles as they issue to make up the nerves, they must be freed from the connective tissue so abundant about and between the roots where they pass through the walls of the cranium and the ganglionic chamber.

In *Bdellostoma* five nerves supply the jaw apparatus. They all leave the medulla cephalad of the ear capsule and, as the snout region extends far in front of the brain, the course of most of these nerves approaches the horizontal. From before, backward, they are:

1. Nasalis
2. Ophthalmicus
3. Maxillaris
4. Mandibularis
5. Facial

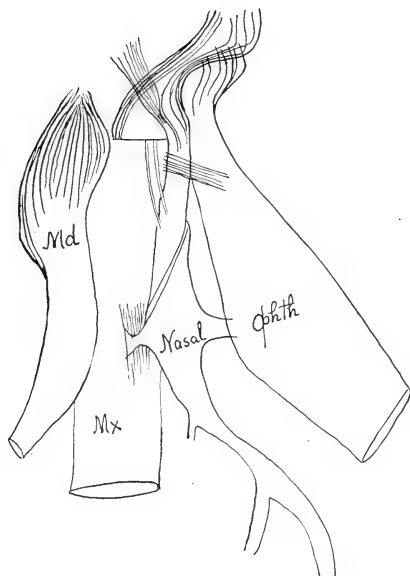


Fig. 31 Ventral view of roots of V nerve of *Bdellostoma*, to show the relation of the root bundles of the nasalis, ophthalmic, maxillaris, and mandibularis leaving the medulla.

The nasalis is the mesial nerve, the ophthalmic next, and so on. The nasalis is therefore the most anterior nerve of the group (figs. 29 and 36). It is the nasal nerve par excellence, being largely devoted to the hollow nasal apparatus, both inside and outside. The nerve supply to the structures of the outer walls is largest, but perhaps not most important, for the nerves to the structures placed inside the nasal capsule and tube

innervate not only the general mucosa, but a branch is supplied to each olfactory plate which enters the olfactory membrane along the dorsal aspect of the olfactory nerve bundle supplying

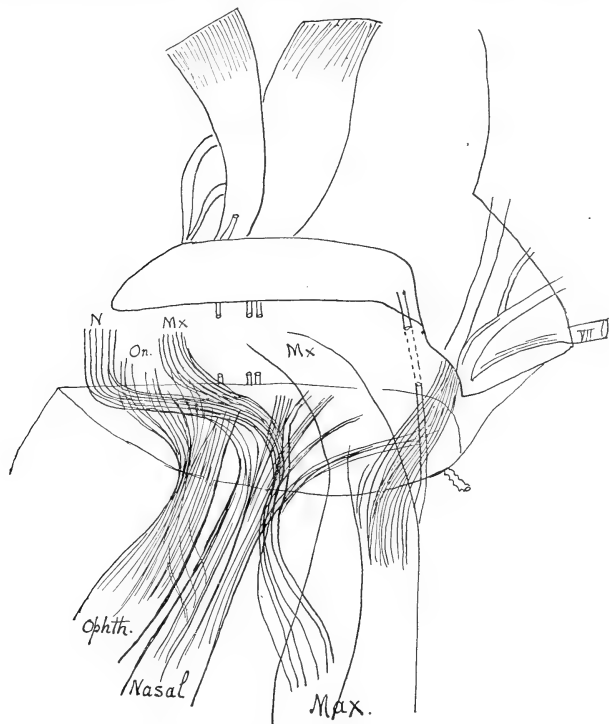


Fig. 32 View of the dorsal surfaces of the nasalis, ophthalmicus, and maxillary roots of *Bdellostoma* seen from the ventral surface of brain. The roots are dissected out and turned back, thus exposing their dorsal surfaces. The root of the nasalis receives the mesial bundles from the medulla.

the plate. The nasal nerve sends an important branch to the roof of the mouth, which palatine branch caused this nerve to be named the palatine, and since it is not visible until it breaks

through the connective-tissue root sheath on the ventromesial face of the larger maxillomandibular nerve trunk, it has been described as a branch of the latter.

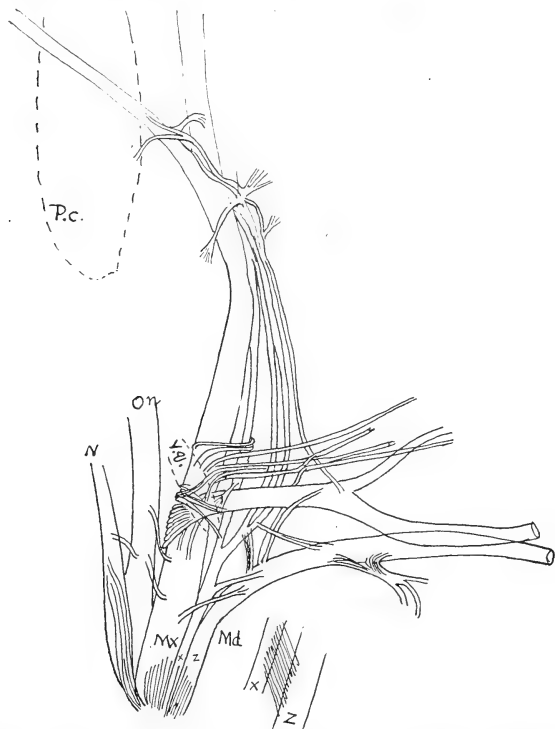


Fig. 33 Ventral view of the left trigeminus of *Bdellostoma*, showing some of the anastomoses of the root bundles and the manner of origin of the maxillaris and mandibularis.

Most of the root bundles of the nasalis nerve arise (figs. 31 and 32) from the mesial part of the tip of the fork of the medulla, cross ventrad of the root bundles of the ophthalmic and turn sharply to enter the base of the nasalis root. Other bundles

issue direct from the medulla in line with the axis of the nasal trunk, still another group of root bundles arise laterad of the root and curve mesad to enter the root of the nasalis. The nasalis is not a branch of any of the other roots, but a distinct and important nerve certainly equal in morphological value to the ophthalmic. Its position ventrad and sometimes laterad of the ophthalmic is due to the crowding of the parts of the trigeminal complex into such narrow quarters that the fiber bundles leaving both the mesial and lateral borders of the medullary projection are both crowded ventrad on both edges, and curved inward below the medullary lobe.

These root bundles unite into a cylindrical trunk which bends at once mesad or toward the brain running cephalad along the ventral border of the brain, nasal capsule, and nasal tube. It gives off small twigs to the fat-body inclosing it, then a large anastomosis to the ophthalmic near where the optic nerve passes outward between the two. The optic nerve passes dorsad of the nasalis, but ventrad of the ophthalmic, and the nasalis and ophthalmic exchange fibers at the cephalic end of the ganglion, and both give off a small number of fibers to the optic nerve and its fat-body where it passes between them. It thus reaches the nasal territory mesad of the ophthalmic and thus lies mesad of the ophthalmic for the larger part of its course. The terminal branches of the nasal division of the nerve in the anterior snout region are mainly close to and upon the nasal tube. The palatine division of the nerve ends in a brush of terminal branches which supply the mucosa of the roof of the mouth, the median tooth and from this region forward anastomose with the nerve veil and network of the snout.

A bird's-eye view of the nasalis nerve may be given as follows: It is the mesial root and thus the most anterior nerve of the jaw complex. It anastomoses with the ophthalmic nerve at its root also where the optic nerve passes between them, both giving off fibers which join the optic nerve trunk. It sends branches: 1) to the fat-body covering the V root; 2) to the optic nerve and fat-body; 3) to the posterior end of the nasal capsule; 4) to the lateral fat-body of the nasal capsule; 5) to the ventral

floor of nose, dorsal wall of the mouth, and to the hypophysial canal; 6) to the fat-body on the ophthalmic nerve; 7) to the sides, dorsal and ventral faces of the nasal tube out to the tip of same; 8) to the roof of the mouth and nasal capsule and nasal tube; 9) five branches to connective tissue and mucosa of the roof mouth and to the palatine tooth; 10) to the nerve net of the jaw apparatus; 11) branches to each nasal fold, companions of the branches of the olfactory nerve in the innervation of the sensory olfactive epithelium.

The nasalis nerve is clearly the most anterior nerve of the trigeminal complex for two reasons. In the first place, it retains its own independent roots of origin from the central nervous system which leave the medulla in part at least mesial to those of the ophthalmic, and, in the second place, because the peripheral distribution of its fibers is to structures lying in general mesad of those supplied by the ophthalmic nerve whose territory lies laterad and consequently morphologically caudad to nasalis territory. It carries motor fibers and innervates the nasalis among other muscles. It is therefore a mixed nerve.

The terms nerve veil and nerve network refer to the nerve structures of the distal portion of the jaw apparatus of *Bdellostoma* which represent the jaw plexuses of *Amphioxus*. The characteristics of this plexiform structure will be given when we come to the branchings of the jaw nerves in and about the snout, buccal rim, and tentacles.

The term fat-body will be used to designate a variety of lipid structures varying from the continuous dermal sheet to large and small, continuous and limited bodies about the nerves on and between the muscles, to others attached to the connective tissue sheets in the interspaces between the organs, about the nasal capsule, the eye, and optic nerve. Usually they receive branches from a passing nerve, with surprising frequency they receive the whole of the terminal branches of relatively large nerves, thus forming an end-organ. They are evidently often more than accumulations of fat in the connective tissue. *Amphioxus* lacks such structures entirely, but they play an important part in the organization of the nervous apparatus of *Bdellostoma*.

The roots of the ophthalmic nerve leave the brain, as shown in figures 31, 32, laterad and dorsad of the mesial bundles of the nasalis. They form the large nerve trunk which holds the mesial position among the nerves leaving the tip of the medulla seen from above, before the roots are dissected out. It soon anastomoses with both the nasalis and the maxillary trunks and runs forward diverging slightly laterad from the sagittal plane. Near the level of the posterior end of the nasal capsule it gives off a superficial branch, sometimes two branches, which rises to the surface of the head just in front of the eye and enters the subdermal lymph space and joins the complex of nerves which ramify throughout the subdermal lymph space, in the skin and over the muscles forming the floor of the lymph space. Some of these ventral branches pass down between and also through the muscles to join the coarse nerve net of the inter-spaces and the loose connective tissue holding the muscle of the mouth in place. Similar branches rise from the deeper branches of the ophthalmic to join the complex and nerve net in the subdermal space (figs. 34 and 35 B). On reaching terminal territory the superficial branch breaks up into small nerves which join the jaw plexus which covers the snout region like a fine-meshed veil (fig. 34).

The main trunk of the ophthalmic runs forward and branches continuously to serve the muscles and other structures it passes, and the branches to the skin and tentacles terminate in brushes of fine fibers (fig. 35A). A large commissure of fibers passes between the ophthalmic and mandibular nerves at A, figure 35. Altogether it is a remarkable bundle of fibers.

The maxillary nerve roots form a large nerve leaving the dorsal lateral edge of the tip of the medulla as seen from above, and its roots are drawn together from a wide territory in the medulla (figs. 31 and 32). It is closely bound up with the mandibular nerve and the anastomoses between the two near their origin from the medulla are extraordinarily extensive and complex, as shown in figure 33. There is great variation in the interchange of fiber bundles. I have selected the drawing shown in figure 33 as on the whole typical of the relation of these



Fig. 34 Dorsal view of a dissection of the left ophthalmicus lateralis, VIIIa, (buccalis) and VIIIb complex in the subdermal space and skin and the character of plexus formation in the tentacular region of *Bdellostoma*. Only a part of this complicated system is shown in the figure. Note the recurrent branches to the aponeurosis and muscles at the anterior end of the nasal capsule.

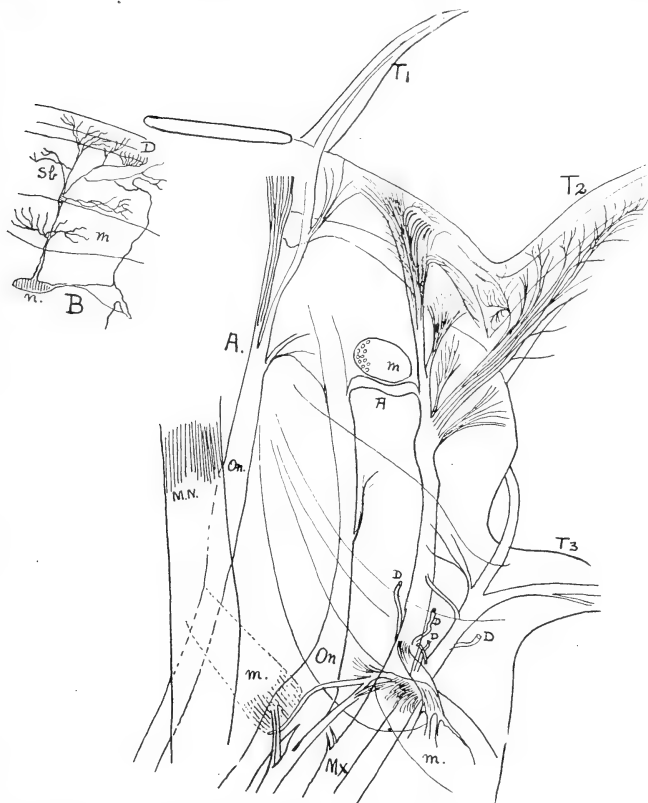


Fig. 35 A. Dorsal view of a dissection of some of the snout nerves of *Bdellostoma*. Forward extensions of the two trunks of the ophthalmic and the maxillary nerves are shown. The branches of the latter to the second tentacle and the skin between it and the first tentacle are drawn in more detail to show the tuft-like terminals. At A is shown an anastomoses between ophthalmic and maxillary nerves in the form of a large bundle of fibers. Near by is seen an exchange of fibers in the form of a small bundle between the two branches of the ophthalmic.

B. Figure to illustrate the connection of the subdermal nerve net with deeper-lying structures. Nerve branches descend through the muscle and rise from nerve net and nerve trunks through the muscle to the surface. The arrangement recalls the association of parts found in the plexiform innervation of the jaw apparatus of *Amphioxus*.

two nerves as they are being made ready for their peripheral distribution.

As the drawing shows—and great care was taken to reproduce accurately the number, relative size, and the course of the nerve bundles present in this individual as seen from the ventral surface—a large addition is made to the mandibular nerve bundles from the fibers which leave the brain in the maxillary trunk. Between *x*, which is a part of the maxillary trunk, and *z*, the mandibular trunk, a thin band of fibers running apparently from the latter to the former, is shown in the small insert at the side. Two anastomoses with the ophthalmic trunk are also shown. The method of branching to the dermal structures is shown in figure 35. The maxillary nerve is also connected up to the jaw plexus in the snout region. In figure 35, at *D*, are shown four considerable branches which run from the trunk direct to the skin. Besides innervating a number of muscles of the jaw apparatus, it furnishes much material to the sensory jaw plexus. It also innervates the velum by at least two branches. It is particularly the nerve of the distal portion of the jaw apparatus, both motor and sensory, sharing with the ophthalmic in this control. It shares with the VII the control of the hyoidean apparatus.

The mandibular nerve is not gathered into a single trunk in any part of its course. The main part of it leaves the medulla laterad of the maxilla, is closely applied to it and tied to it by anastomoses.

Another part leaves the trunk of the maxillary nerve as a thin band of fibers issuing from the dorsomesial surface near the edge and wrapping around the ventral face of the trunk to run laterad across it to join the main nerve, as shown in figure 35. One division of the veloquadratus muscle is soldered to the maxillary nerve at this place (fig. 35, *v.q.*). The mandibular innervates the dentigerous jaw or basal portion of the ancestral jaw bar, also the mandibular muscles, both retractors and protractors, and the muscles controlling the anterior part of the hyoidean apparatus or the jaw-supporting mechanism, as well as supplying the skin structures of its territory. As figure

35 indicates, the fibers of the mandibular nerve are made up into discrete bundles very soon after leaving the brain, which are destined for distinct peripheral structures and run out to them without further complications; i.e., its main branchings occur close to its origin.

From the foregoing sketch of the maxillary and mandibular nerve trunks it is quite apparent that these nerves were present long before the dentigerous jaws arose out of the Amphioxine jaw apparatus and that they have been gradually transformed, as the jaw apparatus evolved, into the characteristic nerves of the maxillary and mandibular mechanisms of the higher vertebrates, gradually assuming the condition and appearance of nerves devoted almost exclusively to these important structures. In *Bdellostoma*, as the mandibular mechanism has already been established and the maxillary mechanism is still undifferentiated, the nerves of the former show greater specialization for the control of a motor mechanism than does the maxillary nerve. Neither the maxillary nor the mandibular nerves are new branches of old nerves, they are segmental trunks more ancient than definitive gnathostome jaws.

The fifth member of the trigeminal complex is the so-called seventh of vertebrate anatomy otherwise known as the N. facialis. It connects with the lateral border of the medulla a short distance behind the group of four which leave the tip. After viewing the crowded condition of the roots on the tip of the medulla this interspace, bare of nerve trunks, is noticeable. The manner of connection of the VII roots with the edge of the medulla varies. Sometimes it appears as a single trunk; usually it shows two roots, one motor root from the ventral edge of the medulla and an acoustic root more dorsal. Occasionally a third fine strand of fibers leaves the medulla cephalad of the motor root and runs out some distance before it joins the nerve trunk. However, these two or occasionally three external roots do not indicate the internal complexity of its make-up. Into its root enter fibers from six sources.

Its peripheral course is outward and backward around the auditory capsule, as though the latter by growing forward had

carried the nerve with it. After its curve around the anterior end of the auditory capsule it runs ventrad and laterad, keeping close for a time to the hyoid arch, and then it sweeps forward, in a curve for a distance, on the surface of the muscles just above the lateral border of the hyoidean plate to reach its final termination in the nerve veil about the fourth tentacle. In its course it sends branches to several hyoidean muscles. As it crosses the copulo-palatinus it sends a small branch dorsad into a small muscle (fig. 36, *VII m.*) about an eighth of an inch long and thinner than tissue-paper. This muscle lies closely applied to the surface of the copulo-palatinus. Perhaps it is a vestigial muscle. Its function is not apparent. I have found several structures in *Bdellostoma* which are as non-conformist as this minute muscle and will consider them in a later contribution.

This fifth segmental nerve, the most posterior of the group, does not reach any part of the distal jaw apparatus other than the fourth tentacle and its connections are mainly to the jaw supporting apparatus. It is thus distinctively a hyoidean nerve. It also does not innervate any of the muscles which move the dentigerous jaw nor the mucosa in any part as far as my observations go. In this it differs from the homologue in fishes higher up. It is postvelar and in *Bdellostoma* postmandibular. It has less to do with the structures of the jaw bar than either the ophthalmic, maxillary, or mandibular nerves. Like the nasalis at the anterior end of the series which has specialized in the service of the olfactory apparatus, the facial at the posterior end of the series has specialized in the service of the hyoidean apparatus. It takes part in the formation of the nerve veil, sending its contributions into the net near the fourth tentacle. The connection with the ophthalmicus superficialis noted by P. Fürbringer is one of these net connections, and it is impossible, as I find it, to yet determine what nerve elements the facial connects with, as not only the ophthalmicus superficialis, but also the other nerves of the jaw apparatus take part in the net building. The nearest trunk to the facial in this locality is what Miss Worthington has called the nerve VIII a, and Allis the buccalis. This nerve runs out below the eye, i.e., posterior to it and conse-

quently ventrad of the ophthalmicus lateralis on the side of the snout. It will take more study and different methods from any yet applied to differentiate the elements entering the nerve veil of the snout so that they may be followed back to the parent roots.

Having briefly described the origin and interconnections of the roots of the jaw nerves and their course to the periphery, it is in order to explain how they intercommunicate and are tied together at the periphery. This anastomosing of peripheral branches of the main nerve pathways is apparently for the purpose of shunting impulses by several paths toward the brain centers and permitting a diversified radiation of motor and other impulses toward the periphery for one or several choices out of the many possible combinations of peripheral reactions. This veil is worthy of physiological study. Since time after time new dissections have disclosed additional interconnections, I am far from saying that the following account is complete. I present these results of my dissections to serve as a basis of further study. A general view of the peripheral relations may be given in few words. The snout region of *Bdellostoma* in and under the skin is veiled over by a wide-meshed network of anastomosing branches of the nerves of the jaw apparatus (figs. 34, 35, 36). This net is located in the skin and between the skin and outer muscles of the head. It is most highly developed in the region between the eye and the tentacles. It is connected with numerous anastomoses, penetrating all parts of the snout, forming here and there special net coverings, e.g., the network about the anterior section of the velo-quadratus.

The nerves contributing branches to the network associated closely with the eye and nose complex are:

1. Nasalis
2. Ophthalmicus profundus
3. Ophthalmicus lateralis
4. Acusticus a
5. Acusticus b
6. Mandibularis
7. Maxillary group
8. Facial

What the distribution of these several elements through the net is, has not been made out. It is notable that so many brain centers have established connection with the sensory outposts of the anterior end of the head, and that the muscles of the jaw apparatus are closely tied up with this mechanism, as also are the eye and nose.

TO SUM UP THE NERVES

In *Amphioxus* the five nerves of the jaw apparatus arise segmentally and evenly spaced from a long section of the central nervous system and run to the walls of the buccal chamber, the jaw apparatus and its tentacles, the velum and the cheeks and lips of the buccal region. Here they form nets or plexuses, the most extensive of which surround the jaw bars and their muscles and extend part way out on the tentacles. The nerves from the right and left sides also anastomose freely. Thus the parts of the jaw apparatus and the walls of the buccal cavity are tied together as a physiological or operating unit, and this extensive peripheral intercommunication may be an expression of the lack of a similar central mechanism and an effort to make good the deficiency. The jaw nerves are thus tied together peripherally more intricately than we yet know them to be centrally. The whole of the jaw apparatus is used in the work of food collection more or less passively.

In *Bdellostoma* the origins of the five nerves are crowded together by the condensation of the long segment of the spinal cord of *Amphioxus* into a short section of the spinal brain, the medulla. They all leave a projecting lobe of this spinal brain with their root bundles more or less intermingled, but still recognizable as separate nerves. They all run to the jaw apparatus, which has been separated into two distinct parts, one of which is put into active use in food gathering in a predacious manner, and the rest of the skeleton of the jaw apparatus is left for the passive work of a supporting skeleton for parts of the mouth having little motion.

Arriving in the territory of the jaw apparatus, the nerves intercommunicate by extensive anastomoses to build a plexi-

form veil and smaller nets with their connecting strands, enclosing the structures in an Amphioxine plexiform nerve apparatus, the character of which leaves no doubt of its ancestral origin.

The labial cartilages of the cartilaginous fishes have attracted the attention of anatomists from the time of Cuvier, who held that they are related to the jaws and represent the maxillary and intermaxillary, among other bones of the fish head. J. Müller held that they were aberrant structures and had no relation to the jaw structures or any part of the mouth. Gegenbaur thought they were remnants of premandibular gill arches. It has been an open field for guessing. The latter view seems to be the one in favor, notwithstanding the fact that no traces of gills or nerves that could belong to such body segments have been found.

Excepting *Ammocoetes* and the Myxinoids, the Chimaeroids have preserved the Amphioxine jaw apparatus more completely than any other of the cartilaginous fishes. *Callorhynchus* and *Chimaera* serve to bridge over the gap between the Myxinoid and what we are pleased to call the Gnathostomes. In figure 10 the relations of the parts of the skeleton of the jaw apparatus of *Callorhynchus* are shown, and in figure 30 those of the related *Chimaera* are displayed, both from the left side, for ready comparison with that of *Bdellostoma* (fig. 25). It will be noted that in *Callorhynchus* the mechanism is about as complete as in *Bdellostoma*. It consists of two well-defined cartilaginous jaw bars, marked 3, fastened to the hyoid below and just outside of the mandible, 3b. They run forward and upward to the sides of the nasal region, 4 and 8, where they are connected with the tentacular cartilages grouped lateroventrad of the nasal capsules. The tentacular cartilages of the third and fourth tentacles of *Bdellostoma*, which are placed opposite the middle and lower part of the buccal aperture (figs. 22 and 25), are here located with the cartilages of the first and second tentacles close about the nasal apertures, and they no longer extend outward as slender tentacular rods but lie in flaps of the skin and the large rostral snout. As in *Bdellostoma*, the distal part of the

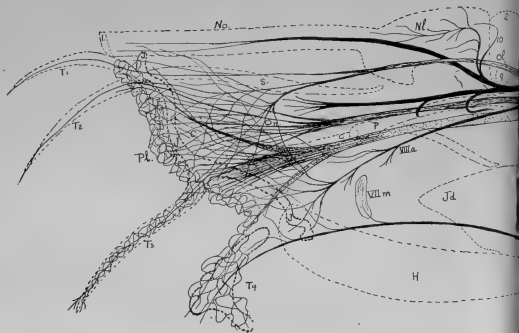
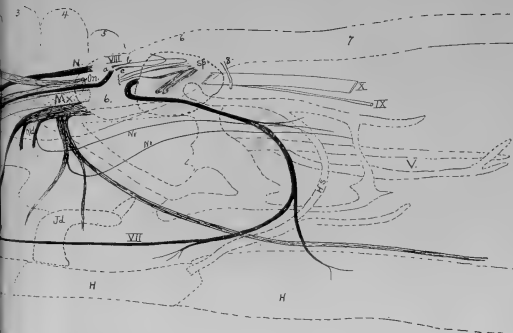


Fig. 36 Composite picture to illustrate the amphioxine condition of the innervation of the jaw apparatus of *Bdellostoma*. The nerves are sketched in over a dotted outline of the major part of the head skeleton. These fundamental facts are illustrated: 1) The position of the buccal cavity (oral hood cavity) between the buccal aperture and the velum. The innervation of the latter. 2) The persistence of an extensive amphioxine jaw apparatus composed of jaw bars with the proximal dentigerous jaws disconnected for free action, and tentacle bearing distal sections of the bars framing in the buccal aperture as in *Amphioxus*. 3) The plexiform innervation of the tentacular portion of the jaw apparatus forming the snout. 4) The enormously developed hyoidean jaw sup-



port. 5) The course of the five nerves controlling the jaw apparatus, forward and ventrad, carrying motor and sensory fibers for the innervation of jaw apparatus and its enclosed buccal cavity. 6) The branches of the nasalis innervating the olfactory mucosa together with other features of innervation acquired since passing the *Amphioxus* stage, such as the lateralis nerves. 7) The caudad displacement of the velar folds with the muscles which operate the velum remaining in their original territory near the mandibular nerve trunk.

The significance of the numbers is as follows: 1, terminal nasal cartilage; 2, olfactory lobe; 3, forebrain; 4, midbrain; 5, cerebellum; 6, medulla; 7, spinal cord; 8, first spinal nerve; 9, eye; 10, olfactory branches of nasalis nerve.

jaw bar is separated from the mandible or basal part and has the same attachment, viz., to the hyoid, not at the tip, but further back, as shown at *b*, figure 10. This distal part is composed of seven pieces of cartilage on each side of the head; the basal piece is strongly attached by tendon to the dorsal edge of the hyoid and bends upward and forward to connect midway to the nose with the second segment, 4, the body of which is sickle-shaped, curving forward and inward to its attachment to 5, at the side and in front of the nasal capsule. Segments 3 and 4 span the distance between the ventral mandibular territory to the nasal region and the maxillary territory. Segment 4 has a prong which leaves the curve of the sickle about midway and runs up, back and inwards, to attach at the side of the skull. Segments 6, 7, 8, and 9 are, respectively, the equivalents of the first, second, third, and fourth tentacles of *Bdellostoma*. The cornual and subnasal cartilages do not seem to be represented as separate cartilages in *Callorhynchus* unless the cartilage of the median nasal fold is the remains of the subnasal bar of *Bdellostoma*. The long nasal tube of the latter is reduced to the capsular parts, which in *Callorhynchus* are well developed as two deep cartilaginous pockets set on either side of the median nasal cartilage. *Callorhynchus* has in addition a large long median dorsal snout cartilage which extends forward into the fleshy snout. The two cartilages of the first and second tentacles extend into the snout along either side of and somewhat ventrad of the median snout cartilage.

The large hyoidean support cartilage lying below the mandible in *Callorhynchus* was called by J. Müller a second lower jaw, and he held it to be a peculiar cartilage not belonging in the vertebrate plan of structure, in which category he also placed the lower labials of *Callorhynchus* and other cartilaginous fishes. G. B. Howes homologized the labials of *Callorhynchus* with the mouth supports of *Bdellostoma*, but failed to see the relations of these parts to the mandibular cartilages and did not understand the morphological nature of this apparatus. His homologies are correct only in part.

The relation of the parts in *Chimaera* is shown in figure 30. They are reduced in number in this species and as a whole fall

short of preserving the Myxinoid stage of the primitive jaw apparatus. They present us with a stage of reduction lying between *Callorhynchus* and the *Elasmobranchs*, and the parts in *Chimaera* are important for that reason.

The series of facts presented shows the genetic relationship of the skeleton of the jaw apparatus step by step from *Amphioxus* to *Ammocoetes* to *Bdellostoma* and to the *Chimaeroids*, which are accepted *Gnathostomes*. It follows that the associated structures, such as muscles, nerves and blood vessels are likewise genetically related. As the nerves are dominantly associated with the jaw apparatus, an examination of them should disclose some indication of this genetic relationship, as after the skeleton they are usually less changed during the course of phylogenetic development than either the muscles or the vascular supply. The results of such an examination of the nerves of *Bdellostoma* have been presented in the foregoing pages. I believe that the two conclusions given at the beginning of this contribution are therefore fully supported by the evidence submitted.

It is also evident that the lower jaw or mandible is the first part of the biting jaws to be established. The maxillary structures arose in response to the stress and pressures developed as the *Bdellostomid* mandible (which on first sight appears to operate largely in a horizontal direction, but which develops its main stresses while in a vertical position outside the mouth) acquired relations such that these vertical stresses were developed with the jaw inside the mouth. With the jaw in action, the hyoidean apparatus of *Bdellostoma* takes up the vertical stresses developed in the movements of the mandible, through its muscular and tendinous attachments to the skull above it, especially the cartilaginous frame which includes the quadrate, palatine, and other elements of the skulls of higher forms. But the hyoidean stresses have not brought forth a definite maxillary anvil to take the pressures of the mandibular hammer. They have, however, brought forth a chondral condensation in the membranes tying the hyoidean base plate to the skull. In the *Chimaeroids* the skull has solidified into cartilage in a large way; only remnants of the membranous skull remain, all the separate cranial

elements of *Bdellostoma* being fused together in a cartilaginous unit.

A maxillary region is marked out by the presence of teeth and is, however, limited behind by the articulation of the mandible with the base of the skull. The hyoidean base plate is still present in the form of a large support plate below the mandible, and we are thus supplied with an instructive intermediate stage between the Myxinoids and the sharks in which the Amphioxine jaw apparatus is still present in its modified Myxinoid form with a well-defined mandible underlaid by the old time Myxinoid hyoidean support. The parts of the distal section of the ancestral jaw apparatus are not fused together nor are they fused with the cranium. But a new relation of parts has appeared—the mandible is articulated to the base of the cranium, being tied to its fulcrum by strong tendons. From this time on the distal section of the jaw apparatus fades away, the hyoidean apparatus undergoes reduction, the second mandible disappears and the newly acquired maxillomandibular articulation becomes the seat of stresses which call forth many different mechanical devices in the effort to improve the biting jaws and to adapt them to special conditions of food gathering and defense which are constantly arising in the efforts of animals to adjust themselves to their environment. The conditions of life become complicated and survival more difficult and the differentiation of the biting jaws is an expression of this struggle. We thus see that *Bdellostoma* has not lost definitive maxillary cartilages because they were not developed in vertebrates until the Myxinoid stage was passed. However, the fundamental cartilages of the head framework with which the maxillary mechanism is associated and to which they are more and more mechanically united as we ascend the vertebrate scale are well developed. The pulls and stresses set up in the skeletonous tissues by the movements of the head parts have caused the appearance of cartilage and chondral tissues in those localities most needing support and those which form the foci of the mechanical stresses due to the pull of the contractile tissues, until in *Bdellostoma* the basic cranial cartilages found in the vertebrate stock above the Myxinoids in great and increasing variety and flux, have been built up into a head

skeleton of considerable extent; but as related to vertebrates above the Myxinoids it represents only the early stages in the ontogeny of their head skeleton. This is as one would expect.

Therefore the development of the craniofacial apparatus in *Bdellostoma* is not an abnormal feature of vertebrate morphology either from the viewpoint of palingenesis or ontogenesis. It is in harmony with the main current of cause and effect which we call evolution of structure.

This is clearly shown by a comparison of the musculature, nerve and vascular supply and the distribution of the connective-tissue sheets and tendons, indicating the directions of the lines of stress and the location of future structural changes, for where stresses are developed new structural arrangements are evolved and hard parts come into being to take up and transmit the major stresses.

As the embryo of *Bdellostoma* is flattened between egg-shell and food yolk, there is no possibility of development in full form as occurs in the free embryo of *Ammocoetes* and larvae of *Petromyzon*. The jaw mechanism is flattened and vertically compressed under the overgrowing brain, but the early larval stages show that it grows cephalad and soon projects beyond the anterior end of the brain. As the food yolk is absorbed and a place made for the growing head, the parts expand dorsoventrally as well, the snout region projecting into the space left free by the absorption of the yolk. The jaw mechanism passes through the *Amphioxus* and *Ammocoetes* stages, which are functional structures in these animals in early embryonic life, by a series of rapid transformations so that the structural characters of the ancestral stages represented by *Amphioxus* and *Ammocoetes*, while marked out, do not progress beyond embryonic tissues and by the time definitive cartilage and muscle tissue appear the characteristic structure of the Myxinoid jaw mechanism has been established. There is apparently no remnant of a latent period, such as occurs in the larval *Petromyzon*, even if such a larval state ever formed a part of the life-history of the ancestral Myxinoids.

The jaw bars occupy the same relative position about the mouth of the *Bdellostoma* embryo as in *Amphioxus* and *Ammocoetes*. From the basal part the jaw bars pass out and forward,

tapering as they go, to meet in front of the mouth behind and below the nasal aperture. The tentacles bud out from the bars. They are four in number in nearly all stages after they appear and it is uncertain if more are formed, although there are indications of two or three similar buds near the nasal end of the bars. They do not develop far, even if they are tentacular buds, and soon disappear. The basal section of the jaw bar is broad and the two attach to the hyoidean base plate in the earliest stages I have dissected.

We thus find, even in early larvae, that the jaw apparatus has the characteristics of the adult. It is desirable to carry the study of the jaw structures back to their first appearance as differentiated organs.

A few words about the brain.

That section of the spinal cord of *Amphioxus* from which the five nerves of the jaw apparatus issue forms in *Bdellostoma* the anterior part of the medulla. As a result of the growth of the brain vesicle in the descendants of *Amphioxus* this medullary territory has been formed out of the symmetrical spinal cord by the pressure of the posterior part of the brain wall in such fashion that the lateral walls of the spinal cord have been spread apart with the extended and thinned dorsal wall stretched between their edges. The ventral wall of the cord, being thick and resistant, is not disrupted, remaining intact. The brain crowding back into the V-shaped space thus formed has slid backward over the ventral portion of the cord, leaving it unaffected. This process can be observed in the ontogeny of many living forms. An excellent series of figures illustrating the effects of the mechanical stresses produced by the increase in volume of the forebrain vesicle is given by Kerr for *Lepidosiren*. Of course, in the higher vertebrates the process cannot be seen, or up to date has not been seen in full detail, as the nerves leaving the medullary segments are already much crowded when recognizable.

Nevertheless, the medullary region is at first a cylinder and is later split open on top to form the medullary V. In all cases the trigeminal complex leaves the prongs of the V, which usually form terminal lobes. At all times during this growth the brain and cord are under tension, due to being inclosed in their en-

velopes and surrounded by the growing tissues of the body. The spreading walls of the medulla meet with resistance to their lateral travel, since they must displace other structures to make room for themselves. Crowded back from in front by the growing brain, they expand laterally against the resistance of the structures laterad of them. Since in the growing organism, as elsewhere, the forces of reaction are equal to the forces of action, the effect of the lateral pressures will inevitably tend to compress the wall of the medulla. We find evidence of this pressure in the relation of the nerve roots to the tip of the medulla. If in the transformation of cord into medulla only the pressure of the brain in front had been operative, the nerve roots would be found leaving the edge of the arms of the Y on the same level and along a line curving about the rounded anterior end of each arm. However, we find the arms have by lateral pressure been buckled upward and their inner and outer edges, originally anterior and posterior limits, bent ventrad, the inner edge curving laterad and the outer edge curving mesad. The root of the nasalis (palatine) nerve is thus forced below the inner edge of the arm and the ophthalmic occupies the apical position on the edge of the tip of the arm. On the outer edge of the arm we find the mandibular similarly curved downward and appearing to arise from the ventral and lateral surfaces of the arm. The maxillary trunk dominates the field on the dorsal surface of the arm.

Up to the present only facts favorable to the view of the genetic connection of the jaw apparatus of *Amphioxus* with that of the *Marsipobranch* and through the latter with the true *Gnathostomes* have been brought forward. Are there any facts that do not harmonize? It would not be a problem awaiting solution if there were none, and furthermore, when one can work out the solution of a problem in comparative anatomy and get all the facts and leave nothing unexplained or unaccounted for, the millennium will have arrived and comparative anatomy will probably lack incentive.

Some investigators will raise the objection that the m. transversus abdominis of *Amphioxus* is not homologous in its anterior part with the circular muscles of the jaw and velar territory of *Ammocoetes*, for example. I think they are, as I shall explain

in a subsequent contribution. But even though it could not be shown that they are homologous, how much will that uncertainty detract from the body of fact which shows that the more or less longitudinal muscles of the jaw of *Amphioxus* arise out of the transversus, that the transversus is innervated by the nerves of the jaw apparatus, that in *Ammocoetes* these muscles, although attached to the skull on both sides and continuous across the midventral line, bear the same relations to the jaw bars, are innervated in this territory by the nerves of the jaw apparatus (trigeminus), and further only differ from those of *Amphioxus* in having developed two layers with skeletal tissue between them; that in *Bdellostoma* they are split up into segments of circles and have attachments to numerous cartilages, although they still maintain the same fundamental relation to the jaw-bars and continue to be innervated by the nerves of the jaw apparatus? In the face of such facts, what is a denial of their homology worth?

Take the old-time puzzle—the location of the nerves of *Amphioxus* outside the myotomes and their submyotomic position in all the other vertebrate forms. Even if we cannot here solve this problem, does the fact that, at some time and somehow, the muscle seems to have disappeared from under the proximal part of the nerves of the jaw apparatus invalidate the evidence that nerves supplying homologous structures in *Amphioxus* and *Bdellostoma* are not themselves homologous? In other words, does a non-essential fact destroy the value of an essential fact?

Other problems like these might be mentioned, but none seem to me to constitute valid objections to the conclusions drawn from the facts that have been presented, viz.: That the *Amphioxine* jaw apparatus is the parent structure out of which has been evolved the mandibular mechanism of the *Gnathostome* vertebrates and its hyoidean companion mechanism. That to branchial structures the jaw has no genetic relationship, while to the trigeminus complex it bears the relation of end-organ. The mystery of the labial cartilages dissolves when we recognize their origin, follow their unfolding, obsolescence, and final disappearance.

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Resumen por el autor, Halbert L. Dunn

El crecimiento del sistema nervioso central en el feto humano
expresado por medio del análisis gráfico y fórmulas
empíricas

El crecimiento del sistema nervioso central durante el periodo fetal es semejante al aumento del peso del cuerpo durante esta época. El crecimiento del sistema nervioso central demuestra, mediante un análisis, la existencia de tres subtipos diferentes de crecimiento, a saber: 1) Crecimiento cerebral, el cual es lento y continuo antes del sexto mes de la vida fetal (embrión de unos 30 cm CH) y más rápido desde dicha época hasta el nacimiento; 2) Crecimiento del tallo cerebral y de la médula espinal, más rápido desde el segundo hasta el final del quinto mes que en épocas posteriores; y 3) Crecimiento del cerebelo, muy lento desde el segundo hasta el sexto mes y sumamente rápido durante los cuatro últimos meses de la vida intrauterina.

Translation by José F. Nonidez
Cornell Medical College, New York

THE GROWTH OF THE CENTRAL NERVOUS SYSTEM IN THE HUMAN FETUS AS EXPRESSED BY GRAPHIC ANALYSIS AND EMPIRICAL FORMULAE

HALBERT L. DUNN

Department of Anatomy, University of Minnesota

THIRTY-EIGHT FIGURES

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INTRODUCTION

The growth of the human central nervous system has long been a matter of interest and many studies on this subject have been published, some of the earlier ones dating back almost a century. These investigations have given us a general knowledge of the growth of the brain as a whole from birth to maturity and have furnished us some information regarding the modifications in absolute and relative size of the various brain parts in early post-natal life.

Our knowledge of the growth of the central nervous system in the prenatal period is much less complete. While the numerous studies on the morphogenesis of the brain and spinal cord in the first trimester of fetal life enable us to draw some general conclusions concerning the early prenatal growth of these structures, there are practically no quantitative data available for this period. Data on the later prenatal growth of the brain are more extensive, and a survey of the literature gives about 500 published records of the weight of the brain between the third fetal month and birth. The more important of these collections are those of Rudinger ('77), Brandt ('86), Arnovljević ('84), Michaelis ('06), Jackson ('09), and Valtorta ('09). A small amount of this material has been presented in graphic form by Zangemeister ('11). The only collection of records of the weights or volumes of the various parts of the brain in fetal life seems to be a short series of figures on the cerebrum, cerebellum, and brain stem published by Valtorta ('09).

The spinal cord has received far less attention than the brain, doubtless because of its smaller size and the technical difficulties of its removal. Our knowledge of the growth of the cord in post-natal life is very incomplete, being limited to the data of Pfeister ('03) and Danielbekoff ('85). Information regarding the changes in fetal life is apparently confined to a study by Giese ('98), published as a St. Petersburg dissertation, which is available only in abstract at the present time.

The following study was undertaken with the hope that a part of this large gap in our knowledge of human growth might be spanned by the systematic examination and measurement of the brain and spinal cord in a large series of human fetuses ranging from the second fetal month to birth, and by the treatment of these data by some of the more modern methods of statistical and graphic analysis. The work was done under the direction of Dr. R. E. Seammon, to whom the writer is very much indebted for constant advice and aid throughout the entire study.

MATERIAL AND METHODS

Material

The material used in this study consisted of 156 human fetuses. Fifty-four of these specimens were males, fifty-six were females, and the sex of forty-six was unknown. The specimens ranged from 3.1 to 53.6 cm. in total or crown-heel length and were quite evenly distributed between these extremes. At least one specimen was available for each centimeter interval of crown-heel length and in many of the centimeter intervals below 30 cm. there were three, four, or five specimens. All of the material was selected from a much more extensive series of cases, with a view to securing specimens in which the brains were well preserved; however, a number of brains which were rather soft were used for certain measurements which were not affected by this condition.

The great majority of the specimens were fixed in 10 per cent formalin to which 1 per cent chromic acid had been added in some instances, and all were afterwards preserved in 10 per cent formalin. Five specimens of the entire series were fixed in Zenker's or Müller's fluid and were subsequently preserved in 70 per cent alcohol. All of the material had been in the preservation for six months or more.

The effect of formalin fixation upon the fetal brain must be known in order to interpret the data correctly. It is unfortunate that no comparison has been made between fresh and preserved material. The fresh brain of the fetus is too friable for any delicate, quantitative measurements to be taken upon it. Consequently, fixation was prerequisite to the collection of the material for this study.

The only checks upon the relationship of fixed and unfixed material at our disposal consist of, 1) the determination of the effects of formalin upon the size of the fetal body and, 2) the relation of the curves of fresh brain weight to that of fixed brain volume.

Several investigators have determined the effects of formalin fixation on the fetus. Patten and Philpott ('21) record an

average increase in the crown-rump length of 4.8 per cent in a series of twenty-two pig embryos which had been in 10 per cent formalin for six months. Schultze ('19) reports an average gain in crown-rump length of 2.3 per cent in a group of eighteen human fetuses which had been in 10 per cent formalin for nine months. The average gain in head length and head breadth of the same series was 1.1 per cent and 5.2 per cent, respectively. Calkins ('21) has found that the effect of fixation on the several diameters of the head is very small and that formalin injection causes the greatest part of this by a distention of the superficial tissues of the scalp. The fetal cranium increases, therefore, very little in size when it is fixed by formalin. The brain, on the other hand, is supposed to gain greatly in volume. King ('10) obtained a percentage gain in weight of from 30 to 50 when the brains of several white rats were subjected to formalin fixation. Such an increase in brain weight or volume could take place in the fetus only at the expense of the dural and subdural spaces, since the circumference and the diameters of the fetal head are but little affected. However, these spaces were found to be quite large in most of the preserved specimens in this series. The volume of the brain, therefore, could not have been increased to any great extent.

The relationship of the curve of fresh brain weight to that of fixed brain volume can be compared readily by tables 2 and 3. Table 2 shows the calculated volume of the central nervous system for each 5 cm. C H interval. Table 3 sets forth the empirically determined values for weights of fresh fetal brains collected from the literature. In each respective C H interval the weight is seen to be slightly greater than is the volume. This difference may be caused in part by the formalin fixation, but the greater part seems to be due to the fact that midsagittal sections of the brain were made before volumes were determined and that intraventricular fluid was lost by this procedure. On the whole, therefore, it is doubtful if there is any considerable change of the fetal brain volume upon formalin fixation.

Five cases of the series were subjected to Zenker- or Müller-alcohol fixation instead of formalin. The effects of various

fixing fluids upon brain tissues have been determined by King ('10) and Hrdlička ('06). The work of King was done upon the brain of the rat, and is analogous, in all probability, to the effects of the fixing fluids upon the fetal brain. Calculated from her findings, the absolute values of the five fetal brains subject to Zenker- or Müller-alcohol fixation would be 10 to 40 per cent lower than the brains which were fixed by formalin. Three of the fetal brains in the present series which were fixed by the Zenker- or Müller-alcohol method lie in the C H interval of 5 to 10 cm. and two are in the C H interval of 10 to 15 cm. A glance at figure 2 shows that these cases adhere closely to the central tendency. Hrdlička subjected the brains of twenty-seven mammals and birds to 10 per cent formalin fixation. Ten sheep brains showed an average increase of 13 per cent after one month of fixation; seven brains of birds an average gain of 20 per cent and six brains of various mammals about 24 per cent. He left the brains in 10 per cent formalin for eighteen months, and then found that they had decreased in size to about 92 per cent of their original weight.

From the results of these investigators as well as the data of this series, it is obvious that 10 per cent formalin fixation has a comparatively slight effect upon the volumetric determination of the fetal brain.

Methods of collecting data

The magnitudes and proportions of the various parts of the central nervous system were determined by several different methods—by lineal measurements, by weighing and determining the volume of each part, by making tracings of median sagittal sections, and by photographing the lateral surfaces of the specimens. The details of these methods and the descriptions of the measurements taken will be considered separately.

Lineal measurements. All lineal measurements were made with a steel vernier caliper which could be read accurately to 1 mm. by the major and to 0.1 mm. by the minor scale. They were made with the brain and spinal cord in situ and always represented the shortest distance between two given points.

Weight. The ponderal determinations were made on a scale accurate to 0.01 gram. Before weighing in every instance the brain part was stripped of its meningeal coverings and was placed on a pad of dry gauze for 30 seconds to remove approximately the same amount of surplus fluid in each case. Weights were always taken upon preserved material.

Volumes. The volumetric determinations were made with a special apparatus constructed according to a plan suggested by Prof. L. W. Jones, formerly of the Department of Chemistry of the University of Minnesota. A sketch of this device is shown in figure 1. It consists of an iron chemical stand (*A*) to which a clamp (*B*) is attached. This clamp holds a hard-rubber stopper (*C*), to which is attached a slender wire (*D*) about 15 cm. in length. A small disk of mica about 1 cm. in diameter slides freely on this wire. The wire is suspended in a jar or wide-mouthed bottle (*F*) which has a spigot at its base and which is partially filled with water. In using the apparatus the water in the bottle is drawn off until the mica disk which floats on its surface touches the hair line on the wire. The mass to be measured is then placed in the bottle and the level of the fluid rises, carrying the disk with it. The water is then drawn off into a beaker of known weight until the mica disk has sunk again to the level of the hair line on the wire. The beaker with the contained water is then weighed and the known weight of the beaker is subtracted from the total, thus giving the weight of the water displaced by the brain part. After the proper temperature correction, this value can be converted into a measure of volume. With practice and proper precautions, it is possible to determine small volumes quite accurately with this apparatus. When working with small bodies, it is desirable to use a bottle with a capacity of not over 30 to 40 cc. A difference of approximately 0.1 cc. in volume can be determined without trouble if a small container is used.

The lineal, volumetric, and ponderal determinations made were as follows:

1. CH: Crown-heel length, or total body length; from the vertex to the tip of the heel.
2. CR: Crown-rump length, or the sitting height; from the vertex to the tip of the coccyx.

3. FO: Fronto-occipital diameter; from the frontal pole to the occipital pole of the cerebral hemisphere. The frontal pole is defined as that point where the anterior, lateral, and inferior surfaces of the frontal lobes meet. In large brains, in situ, this

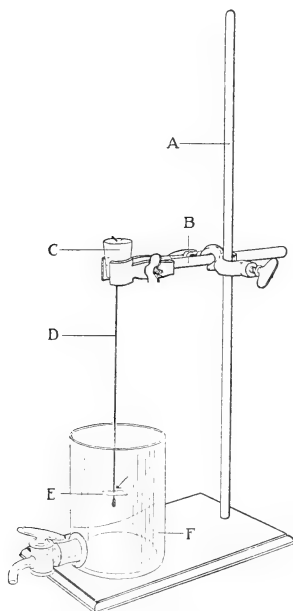


Fig. 1 A figure representing the volumetric apparatus used in the collection of data. A, iron standard; B, clamp; C, stopper; D, wire; E, movable isinglass disk; F, bottle with spigot. The arrow indicates a hair line upon the wire.

point is adjacent to the crista galli. The occipital pole is defined as that point where the posterior, lateral, and the interior surfaces of the occipital lobes meet.

4. TT: Temporal diameter; from the right temporal pole to the left temporal pole of the cerebral hemispheres. The temporal pole is defined as the most lateral point on the anterior

projection of the temporal lobe. This point becomes less definite in large fetuses, but can still be recognized with sufficient accuracy.

5. FS: Frontospinal length; from the frontal pole of the cerebral hemisphere to the point of origin of the first cervical nerve. This measurement was taken to the middle of the body of the first cervical vertebra, for it was sometimes difficult to identify the first cervical nerve because of error in securing exact midsagittal sections of the brain and the spinal cord.

6. SC: Spinal cord length; from the point of origin of the first cervical nerve to the tip of the conus medullaris; the measurement was made with cord in situ.

7. BC: Brain and cord length; the sum of SC (6) and FS (5).

8. PL: Pons length; the greatest inferior length of the pons.

9. CL: Colliculi length; from the most anterior point of the superior colliculus to the most posterior point of the inferior colliculus.

10. VL: Vermis length; the greatest length of the vermis cerebelli parallel with the axis of the pons and medulla.

11. VH: Vermis height; the greatest height of the vermis cerebelli perpendicular to the long axis of the pons and medulla.

12. VSC: Volume of the spinal cord; cord taken from the first cervical nerve to the tip of the conus medullaris and freed of meninges and nerve roots.

13. VPM: Volume of the pons and medulla; pons and medulla cut posteriorly at the first cervical nerve and anteriorly just in front of the pons, meninges removed.

14. VMB: Volume of the midbrain; the midbrain was cut just in front of the pons posteriorly and just in front of the superior colliculi and through the pedunculi cerebri behind the mammillary bodies anteriorly, meninges removed.

15. VC: Volume of the cerebellum; the velum medullaris was severed anteriorly and posteriorly to free the cerebellum from the pons and medulla, meninges removed.

16. VRH: Volume of the right hemisphere, meninges removed.

17. V L H: Volume of the left hemisphere, meninges removed.

18. V B H: Volume of both hemispheres: computed by adding V R H (16) and V L H (17).

19. V E B: Volume of the entire brain or encephalon computed by adding 13, 14, 15, 16, and 17. This method was found to be more accurate in dealing with the soft brains than to take the actual volumes of all the parts, because of the possible loss of brain tissue in handling.

20. V C N S: Volume of the central nervous system; found by adding the volume of the spinal cord (12) to the entire brain or encephalon volume (19).

The ponderal determinations corresponding to the volumetric measurements were taken and are included in table 41.

Methods of treating data

While the accuracy of data of the kind here presented depends upon the nature of measures employed and the care with which these measures are determined, their significance can only be ascertained by the use of a variety of methods of graphic and numerical analysis. A number of these methods have been used in the present study. They may be arranged in order of procedure in five main groups, namely: 1) Construction of field graphs with points of central tendency and establishment of preliminary curves by inspection. 2) Reduction of these preliminary curves, as established by inspection, to empirical formulae based on total body length, and construction of primary data tables. 3) Calculation of values at 5-cm. intervals of body length, calculation of absolute and percentage increments for each 5-cm. interval of body length, calculation of newborn ratios for each 5-cm. interval of body length, and expression of these values in the graphic form. 4) Conversion of the various values as determined by empirical formulae from functions of the body length to functions of the age in fetal months by interpolation on the basis of Mall's ('12) tables of crown-heel length in fetal life. Determination of the monthly percentage and absolute increment on the basis of these conversions; graphic expression of these

values. 5) Determinations of the relative weights of the various parts of the central nervous system as compared to that of the encephalon. These several methods of analysis will now be considered in more detail.

1. *Construction of field graphs and establishment of preliminary curves by inspection.* The data secured for each measurement of the central nervous system were first plotted on a field graph in which the length of the body (C H) was used for the abscissa and the measurement in question for the ordinate. The material was then divided into classes on the basis of 5-cm. intervals of crown-heel length and the arithmetic mean or average and the median were determined for each of these classes. These means were then indicated in the graphs by special symbols, their exact position being determined by weighting for the distribution of the cases according to crown-heel length in the 5-cm. intervals. A preliminary curve was then drawn by inspection on the basis of the combined evidence furnished by the position of the arithmetic mean, the median, and the general distribution of the cases. This method has distinct advantages over the practice of establishing curves of inspection on the basis of the arithmetic mean alone, particularly in those instances where a curve is rising rapidly in a single interval or where the cases are not regularly distributed. The use of both the arithmetic mean and the median enables one to correct for chance deviations in the mean alone with considerable confidence, particularly when the cases are all spread before one on the field graph.

2. *Reduction of preliminary curves of inspection to numerical expression by means of empirical formulae.* The curve of each value as determined by inspection was reduced to numerical expression in the form of an empirical formula on the crown-heel or total length of the body expressed in cm. This procedure in no way increases the reliability of the curve as determined by inspection nor does it increase the accuracy of the points upon which it is based, but it has several important advantages, for it permits an accurate and abbreviated expression of the curve, facilitates exact interpolation and the conversion of values into different scales and forms of expression, and, in some cases, aids in classifying the curves as to form.

The empirical formulae expressing the curves obtained from the material are of several types. As certain of the lineal dimensions of the brain are straight lines when plotted against crown-heel length, they may be expressed by the formula:

$$Y = aX + b$$

where Y is the value in question expressed in cm., X is the crown-heel length in cm., and a and b are empirically determined constants.

The growth of other lineal dimensions of the central nervous system is not so simply related to the lineal growth of the body as a whole and the expression of this relation is more complex. But all may be represented by formulae having the general form:

$$Y = (aX)^b \pm c$$

where Y is the value in question (in cm.), X is the crown-heel length of the body (in cm.), and a , b , and c are empirically determined constants, b being a fractional exponent. Curves corresponding to formulae of this type may also be expressed fairly satisfactorily in the logarithmic form:

$$Y = aX + b \log X \pm c$$

which will be recognized as the formula which Hatai employed with great success in the study of the growth of the various organs of the albino rat and which is regarded by him as of fundamental importance as exemplifying the application of the law of Maupertuis to the process of growth. However, the exponential form has been found the more convenient for application in the present work.

The formulae for the expression of the inspected curves of volumes of the various parts of the central nervous system have as their simplest form:

$$Y = (aX)^b$$

which is modified in certain instances to:

$$Y = (aX)^b \pm c$$

and in others to:

$$Y = d [(aX)^b \pm c]$$

In these formulae Y is the value in question in cc., X is the crown-heel length in cm., and a , b , c , and d are constants, b being always an exponent with a value greater than 1.

Having obtained calculated values for the different measurements under consideration, tables of each dimension were drawn up in some detail. These are tables 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 36, 38 of the present publication. In each of these is given the crown-heel length in 5-cm. intervals, the average crown-heel length, the maximum, minimum, and arithmetic mean (average) of the considered measurement in the interval, the calculated mean for the same measurement, and the absolute difference between the calculated and the observed means. Both the calculated and observed means are recorded to the nearest millimeter for length and to the nearest 0.1 cc. for volumes in these tables.

3. *Calculation of secondary tables.* Secondary determinations made on the basis of the above calculations are included in tables 2, 4, 5, 6, 10, 12, 14, 16. These include the absolute value of each measurement, as determined by calculation with the empirical formula, for each 5 cm. of body length from 5 to 55 cm., and the percentage and the absolute increment of these values for each 5-cm. interval between these points.¹ Included in these tables is also the ratio of the calculated value of the measurement at each 5 cm. of the total body length to the calculated value of the new-born. It was assumed that the value at 50 cm. of crown-heel length represented the new-born and the calculated values for the 5-cm. points below 50 are expressed in per cents of this one. The reduction of values to this common scale is of great convenience, since it permits their direct comparison regardless of their absolute magnitudes.

4. *Conversion of the various values from functions of the crown-heel length to functions of the age in fetal (lunar) months.* This was done by calculating the values by means of the empirical formulae

¹The percentage increment was determined in the customary manner. The absolute value at the beginning of an interval is subtracted from the absolute value at the close of the same interval. The result is divided by the absolute value at the beginning of the interval and the quotient thus obtained is multiplied by 100 in order to reduce it to a percentage basis. The percentage increment is the only simple form of expression of relative growth now in use which is at all satisfactory. However, it gives only a rough approximation of the actual rate of growth in a given interval.

from Mall's tables of the relation of crown-heel length to age in prenatal life (Keibel and Mall, *Human Embryology*, vol. 1, p. 199). The results obtained by this procedure are not to be regarded as final, for the exact relation between body length and age in fetal life is still open to question. They are included, however, because they give, in a rough way at least, some concept of the relation of the growth of the central nervous system to time in the prenatal period and because they aid in the interpretation of its percentage increment. Since Mall's table stops at 270 days, it has been extended by graphic extrapolation to ten full lunar months (280 days) and the length at this age (51.5 cm.) was considered as the norm for the new-born in this type of calculation. The monthly percentage increments of the principal volumetric determinations were calculated secondarily from the values obtained in this manner. They are shown in table 42.

5. *Determination of the relative weights of the various parts of the central nervous system in terms of per cents of the encephalon* is an obvious procedure which needs no particular description. The results obtained are shown in table 40.

SUMMARY OF OBSERVATIONS

Growth of the central nervous system as a whole

The curve of the absolute volume of the central nervous system (fig. 2), when based upon crown-heel or total body length, is concave like all other curves of volume growth of the fetal organs. It may be expressed by the empirical formula:

Central nervous system volume (cc.) = $[0.114 \text{ (C H length cm.)}]^{3.34} + 2.0$

The absolute volume of the central nervous system is about 7 cc. at 10 to 15 cm. (C H). This increases steadily to 36 cc. at 25 cm. (C H), and is then deflected upward sharply to reach 337 cc. at 50 cm. (C H).

When calculated according to age in fetal months, the absolute volume of the central nervous system (fig. 34, curve I) is found to be 2 cc. at the beginning of the third month, rising to 36 cc. at the beginning of the sixth month, and to about 340 cc. at birth.

The rate of growth of the volume of the central nervous system as determined by the 5-cm. interval percentage increment (page 413), (fig. 26, curve I) is approximately 120 per cent for the 10-to-15-cm. interval and descends gradually to about 40 per cent for the 50-to-55-cm. interval.

TABLE 1
Volume of the central nervous system

Formula: Volume of the central nervous system (cc.) = $[0.114 \text{ (crown-heel length cm.)}]^{3.34} + 2.0$
(146 cases)

CROWN-HEEL LENGTH		OBSERVED VOLUME OF CENTRAL NERVOUS SYSTEM			CALCULATED VOLUME OF CENTRAL NERVOUS SYSTEM	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5	3.9	0.7	0.1	0.3			4
5 to 10	7.2	2.4	0.6	1.3			12
10 to 15	12.2	8.6	2.1	5.4	5.0	-0.4	21
15 to 20	16.8	22.5	3.0	12.5	10.8	-1.7	16
20 to 25	22.6	49.5	17.4	31.1	25.6	-5.5	19
25 to 30	27.1	65.4	24.7	46.9	45.3	-1.6	12
30 to 35	32.8	144.2	45.8	81.8	83.9	+2.1	18
35 to 40	37.2	222.8	102.2	141.4	126.7	-14.7	17
40 to 45	42.2	256.9	145.3	197.7	187.6	-10.1	12
45 to 50	48.0	412.5	207.0	306.0	294.0	-12.0	7
50 to 55	52.2	414.3	332.3	367.5	388.5	+21.0	8

TABLE 2

Calculated volume of the central nervous system at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	VOLUME OF CENTRAL NERVOUS SYSTEM	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
<i>cm.</i>	<i>cc</i>	<i>cc.</i>	<i>per cent</i>	<i>per cent</i>
5				
10	3.6			1.1
15	8.0	4.4	122.0	2.4
20	17.7	9.7	121.2	5.3
25	35.0	17.3	97.6	10.4
30	62.8	27.8	79.4	18.6
35	103.5	40.7	65.0	30.7
40	160.9	57.4	55.4	47.7
45	237.3	76.4	47.5	70.3
50	336.8	99.5	41.9	100.0
55	462.2	125.4	37.2	137.0

The percentage increment, when calculated for time, is about 600 in the fourth fetal month. This drops to 185 in the fifth month and to 22 per cent in the tenth month.

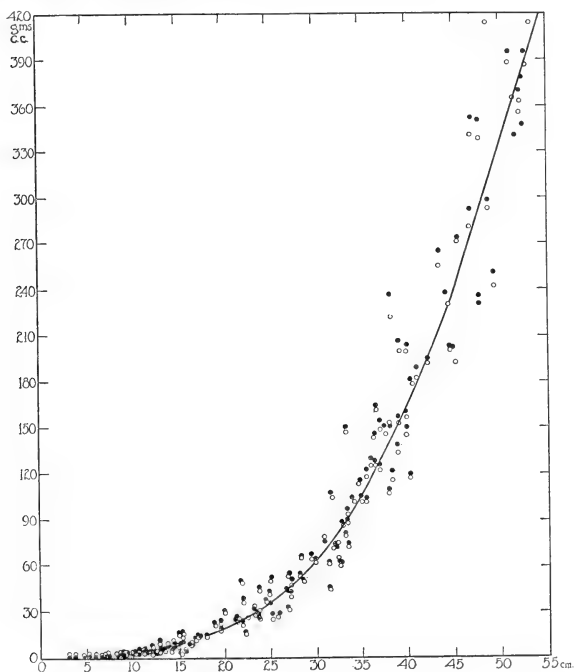


Fig. 2 Field graph and curve of the growth of the central nervous system in fetal life. Abseissa: total body length in cm. Ordinate: central nervous system weight and volume in grams (cc.). Individual cases are indicated by solid dots (for weight) and by circles (for volume). Curve drawn to the formula: $Y = (0.114X)^{3.34} + 2.0$. (Data from tables 1 and 2.)

Growth of the encephalon

The curve of the absolute volume of the entire brain (fig. 3) when based on crown-heel length is a concave curve expressed by the empirical formula:

$$\text{Encephalon volume (cc.)} = (0.125 (\text{C H length cm.}))^{3.16} + 1.5$$

The absolute volume of the entire brain when based on crown-heel length (fig. 3) is about 3.5 cc. at 10 cm. (C H). This increases to 38 cc. at 25 cm. (C H) and then more rapidly to 330 cc. at 50 cm. (C H).

TABLE 3

Encephalon volume

Formula: Encephalon volume (cc.) = $(0.125 \text{ crown-heel length cm.})^{3.16} + 1.5$
(144 cases)

CROWN-HEEL LENGTH		OBSERVED VOLUME OF ENCEPHALON			CALCULATED VOLUME OF ENCEPHALON	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5	3.9	0.7	0.1	0.3			4
5 to 10	7.2	1.8	0.6	1.2	2.2	+1.0	11
10 to 15	12.3	9.5	2.0	5.4	5.4	0	21
15 to 20	16.9	22.4	2.8	11.9	12.1	+0.2	16
20 to 25	22.3	48.8	16.7	30.8	27.1	-3.7	19
25 to 30	27.1	64.7	27.3	45.8	48.7	+2.9	12
30 to 35	32.2	106.3	45.2	76.5	83.0	+6.5	17
35 to 40	37.2	221.8	101.2	132.1	130.1	-2.0	18
40 to 45	42.8	255.1	144.1	191.2	201.5	+10.3	10
45 to 50	48.0	409.4	226.7	299.1	289.0	-10.1	8
50 to 55	52.2	412.3	331.4	364.9	376.5	+11.6	8

TABLE 4

Calculated volume of encephalon at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	VOLUME OF THE ENCEPHALON	INCREMENT IN EACH 5-CM. INTERVAL OF CROWN-HEEL LENGTH		RATIO TO NEW-BORN
		cm.	per cent	
cm.	cc.			per cent
5				
10	3.5			1.1
15	8.4	4.9	140.0	2.5
20	19.6	11.2	133.5	6.0
25	38.1	18.5	94.4	11.6
30	66.6	28.5	74.8	20.2
35	107.6	41.0	61.5	32.7
40	163.3	55.7	51.8	49.6
45	236.1	72.8	44.5	71.8
50	328.9	92.8	39.4	100.0
55	444.0	115.1	35.0	135.0

When calculated to age in fetal months (fig. 34, curve I), the volume of the entire brain is about 1 cc. at the beginning of the third month. It increases to 14 cc. by the first of the fifth month, and reaches the value of 330 cc. by birth.

The rate of growth of the encephalon when determined by 5-cm. percentage increment (fig. 26, curve II) is approximately

TABLE 5

Calculated weight of the encephalon (based on collected data) at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	WEIGHT OF THE ENCEPHALON	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
<i>cm.</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
5				
10	3.3			0.8
15	9.4	6.1	185.0	2.4
20	22.1	12.7	135.0	5.6
25	44.0	21.9	97.5	11.2
30	77.8	33.8	76.8	19.8
35	126.6	48.8	62.7	32.2
40	193.3	66.7	52.7	49.1
45	281.0	87.7	45.4	71.4
50	392.9	111.9	42.4	100.0
55	532.1	139.2	35.4	135.5

TABLE 6

Volume of the encephalon (data of Jackson, '09)
(31 cases)

CROWN-HEEL LENGTH		ENCEPHALON VOLUME			NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean	
<i>cm.</i>	<i>cm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	
0 to 5	1.8	0.4	0.25	0.18	3
5 to 10	7.7	3.6	2.0	2.83	3
10 to 15	12.3	20.0	10.0	13.7	3
15 to 20	17.2	40.0	18.0	27.2	5
20 to 25	22.4	60.0	30.6	45.9	4
25 to 30	27.4	129.0	52.7	85.4	5
30 to 35	32.7	126.7	120.0	123.3	2
35 to 40	38.5	140.0	140.0	140.0	1
40 to 45	44.2	321.3	321.3	321.3	1
45 to 50	45.9	358.0	278.2	322.6	2
50 to 55	51.9	385.0	363.0	374.0	2

140 per cent for the 10-to-15-cm. interval. It descends rapidly to 75 per cent of the 25-to-30-cm. interval, and then more slowly to 40 per cent in the 50-to-55-cm. interval.

The percentage increment of the entire brain volume when calculated against age in fetal months (fig. 28, curve VI) is about 580 per cent in the fourth month, descending to 22 per cent in the tenth month.

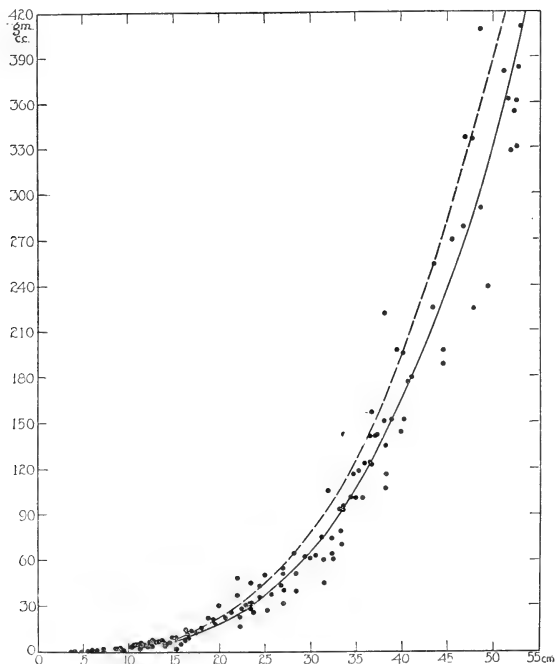


Fig. 3 Field graph and curve of the growth in weight and volume of the encephalon in fetal life (the curve of growth taken from brain weights reported in the literature). Abscissa: total body length in cm. Ordinate: entire brain weight and volume in grams (cc.). Individual cases are indicated by solid dots (for weight). I, curve of encephalon volume drawn to the formula: $Y = (0.125X)^{3.16} + 1.5$. — (Data from tables 3 and 4.) II, curve of encephalon weight based upon data from the literature and drawn to the formula: $Y = (0.13X)^{3.19} + 1.0$ (Data from tables 5 and 6.)

Growth of the cerebrum

The growth of the cerebral hemispheres is portrayed by figures 3 to 9, inclusive, and figures 37 and 38, and is shown numerically in tables 3 to 16, inclusive. It may be expressed by the empirical formula:

$$\text{Volume of the cerebrum (cc.)} = (0.12 \text{ (C H length cm.)})^{3.19}$$

TABLE 7

Volume of the right hemisphere

Formula: Right hemisphere volume (cc.) = $(0.1 \text{ crown-heel length (cm.)})^{3.13}$
(115 cases)

CROWN-HEEL LENGTH		RIGHT HEMISPHERE VOLUME			CALCULATED RIGHT HEMISPHERE VOLUME	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maxi- mum	Mini- mum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5							
5 to 10							
10 to 15	12.5	4.2	1.6	2.6	2.0	-0.6	12
15 to 20	16.9	10.6	2.1	6.0	5.2	-0.8	14
20 to 25	22.6	21.8	9.3	14.2	12.8	-0.4	19
25 to 30	27.1	32.7	12.4	21.8	22.7	+0.9	12
30 to 35	32.6	73.1	21.1	39.2	40.4	+1.2	17
35 to 40	37.2	109.3	48.7	65.8	61.1	-4.7	16
40 to 45	42.5	126.6	67.2	94.6	92.6	-2.0	10
45 to 50	48.0	191.5	102.9	139.0	135.6	-3.4	7
50 to 55	52.2	185.3	148.0	166.0	176.4	+10.4	8

TABLE 8

Calculated volume of the right cerebral hemisphere at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	VOLUME OF RIGHT CEREBRAL HEMISPHERE	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
cm.	cc.			per cent
5				
10	1.0			0.6
15	3.6	2.6	260.0	2.3
20	8.8	5.2	144.0	5.7
25	17.6	8.8	100.0	11.4
30	31.1	13.5	76.6	20.2
35	50.5	19.4	62.3	32.7
40	76.4	25.9	51.2	49.5
45	110.8	34.4	45.0	71.8
50	154.2	43.4	39.2	100.0
55	207.7	53.5	34.7	134.5

The absolute volume of the right and of the left hemispheres (figs. 4 and 5) shows no marked differences—the minor distinctions between the two falling within the margin of experimental error.

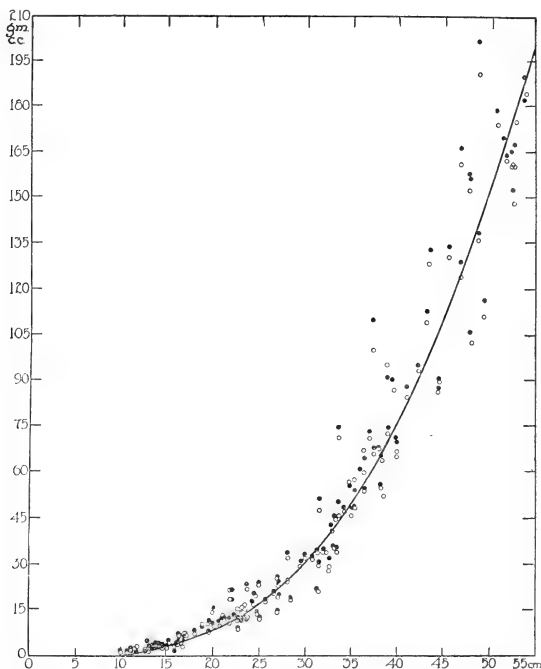


Fig. 4 Field graph and curve of the growth in weight and volume of the right cerebral hemisphere in fetal life. Abscissa: total body length in cm. Ordinate: right hemisphere weight and volume in grams (cc.). Individual cases are indicated by solid dots (for weight) and by circles (for volume). Curve drawn to the formula: $Y = (0.1X)^{3.13}$. (Data from tables 7 and 8.)

The absolute volume curve of the hemispheres shows the same concave form as the volume of the central nervous system (fig. 2). At 15 cm. (C H) the hemispheres are 1.8 cc. in volume, while in

fetuses of 25 to 30 cm. (C H) they have reached an average volume of about 32 cc. After this time the volume curve increases more rapidly, attaining the average of about 300 cc. at 50 cm. (C H).

When examined in relation to age (fig. 34, curve II) the absolute volume of the right cerebral hemisphere is found to be 2 cc.

TABLE 9
Volume of the left cerebral hemisphere

Formula: Volume of the left cerebral hemisphere (cc.) = (0.105 crown-heel length (cm.))^{3.04}

CROWN-HEEL LENGTH		OBSERVED VOLUME OF THE LEFT HEMISPHERE			CALCULATED VOLUME OF THE LEFT HEMISPHERE	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5							
5 to 10							
10 to 15	12.5	4.4	1.3	2.6	2.3	-0.3	12
15 to 20	16.9	10.5	2.3	6.0	5.7	-0.3	14
20 to 25	22.4	20.0	10.2	14.8	13.5	-0.3	16
25 to 30	27.1	30.0	13.0	21.7	24.0	+2.3	13
30 to 35	32.8	50.0	21.0	38.2	42.9	+4.7	18
35 to 40	37.2	89.6	46.8	64.1	62.9	-1.2	16
40 to 45	42.5	110.7	67.0	92.2	94.4	+2.2	10
45 to 50	48.0	188.5	98.5	138.0	136.6	-1.4	7
50 to 55	52.2	192.0	142.0	170.0	176.2	+6.2	8

TABLE 10
Calculated volume of left cerebral hemisphere at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	VOLUME OF LEFT CEREBRAL HEMISPHERE	INCREMENT IN EACH 5-CM. INTERVAL OF CROWN-HEEL LENGTH		RATIO TO NEW-BORN
		cc.	per cent	
cm.	cc.			per cent
5				
10	1.2			0.8
15	4.0	2.8	233.0	2.6
20	9.6	5.6	140.0	6.2
25	18.8	9.2	96.0	12.1
30	32.8	14.0	74.5	21.2
35	52.5	19.7	60.0	33.9
40	78.3	25.8	49.1	50.6
45	111.2	32.9	42.0	71.9
50	154.9	43.7	39.2	100.0
55	200.3	45.4	29.3	129.2

at the beginning of the third month, and this rises at first slowly and then rapidly to about 150 cc. at birth.

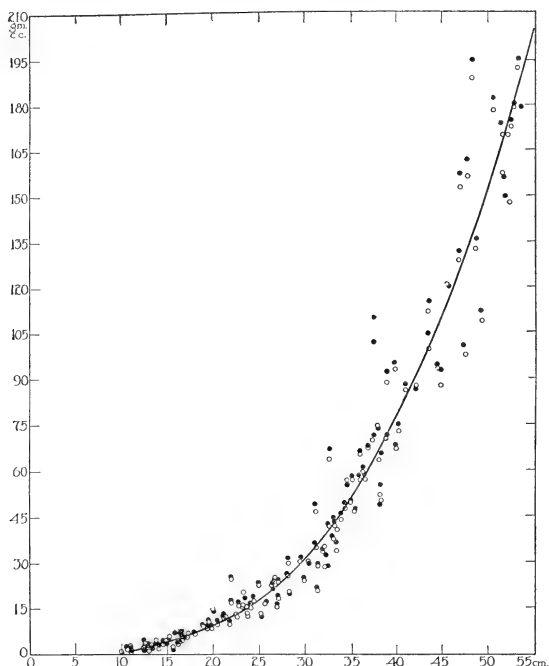


Fig. 5 Field graph and curve of the growth in weight and volume of the left cerebral hemisphere in fetal life. Abscissa: total body length in cm. Ordinate: left hemisphere weight and volume in grams (cc.). Individual cases indicated by solid dots (for weight) and by circles (for volume). Curvedrawn to the formula: $Y = (0.105X)^{3.04}$. (Data from tables 9 and 10.)

The percentage increment of the hemispheres (fig. 26, curve VI) approximates that of the entire brain. It is about 265 per cent for the interval between 10 and 15 cm. (C H) length and steadily declines from this value until it reaches the level of 35 per cent for the 50-to-55-cm. (C H) interval (birth).

The percentage increment of the hemispheres, when calculated by fetal months (fig. 28, curve V), shows an approximate rate of growth of 600 per cent in the fourth fetal month. This rate descends rapidly at first, and then more slowly to about 22 per cent in the tenth fetal month.

TABLE 11

Volume of both cerebral hemispheres

Formula: Volume of both cerebral hemispheres (cc.) = (0.12 crown-heel length (cm.))^{3.19}
(115 cases)

CROWN-HEEL LENGTH		OBSERVED VOLUME OF BOTH HEMISPHERES			CALCULATED VOLUME OF BOTH HEMISPHERES	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5							
5 to 10							
10 to 15	12.5	8.6	2.2	5.2	3.65	-1.55	12
15 to 20	16.9	20.5	4.8	12.2	9.5	-2.7	14
20 to 25	22.4	46.2	18.0	29.4	22.9	-6.5	16
25 to 30	27.1	62.5	25.4	46.6	43.0	-3.6	12
30 to 35	32.8	137.1	56.5	76.1	79.1	+3.0	18
35 to 40	37.2	210.8	95.5	131.4	118.2	-13.2	17
40 to 45	42.3	239.3	134.2	184.8	178.1	-6.7	11
45 to 50	48.0	380.4	201.2	277.0	266.5	-10.5	7
50 to 55	52.2	377.3	302.0	334.4	348.3	+13.9	8

TABLE 12

Calculated volume of both cerebral hemispheres at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	VOLUME OF BOTH CEREBRAL HEMISPHERES	INCREMENT IN EACH 5-CM. INTERVAL		RATIO OF NEW-BORN
<i>cm.</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>	<i>per cent</i>
5				
10	1.79			0.6
15	6.52	4.73	264.0	2.1
20	16.33	9.81	150.0	5.4
25	33.27	16.94	103.0	10.8
30	59.51	26.24	78.8	19.6
35	97.31	37.80	63.5	32.1
40	149.0	51.69	53.2	49.1
45	216.9	69.7	45.5	71.5
50	303.6	86.7	40.0	100.0
55	411.2	107.6	34.5	135.0

The per cent which the volume of both hemispheres forms of the entire brain volume (fig. 7) reflects the relative growth of the other brain parts. The cerebral hemispheres form only from

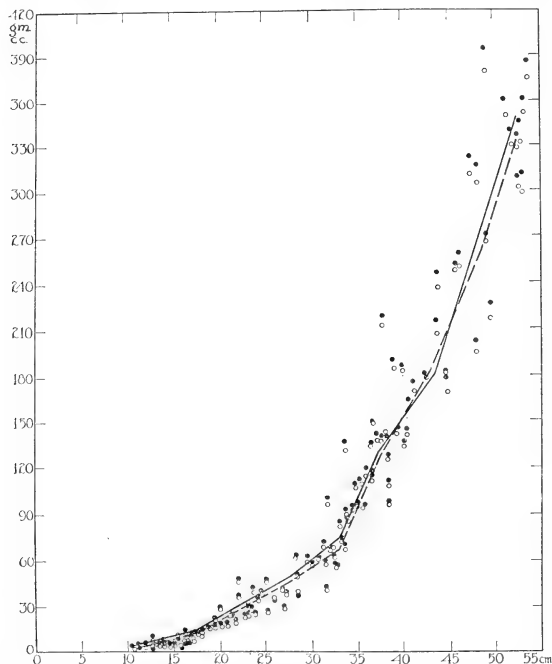


Fig. 6 Field graph and curve of the growth in weight and volume of both cerebral hemispheres in fetal life. Abscissa: total body length in cm. Ordinate: hemispheres weight and volume in grams (cc.). Individual cases indicated by solid dots (for weight) and by circles (for volume). Curve drawn to the formula: $Y = (0.12X)^{3.19}$. (Data from tables 11 and 12.)

88 to 90 per cent of the entire brain volume in the third fetal month. This proportion increases rapidly to between 94 and 95 per cent during the sixth fetal month, but after this time it descends to about 91.5 per cent at birth. This decline is due mainly to the tremendous relative growth of the cerebellum.

The two linear measurements of the cerebral hemispheres (the fronto-occipital and the temporal diameters) also furnish some information concerning the growth of this part of the brain. The

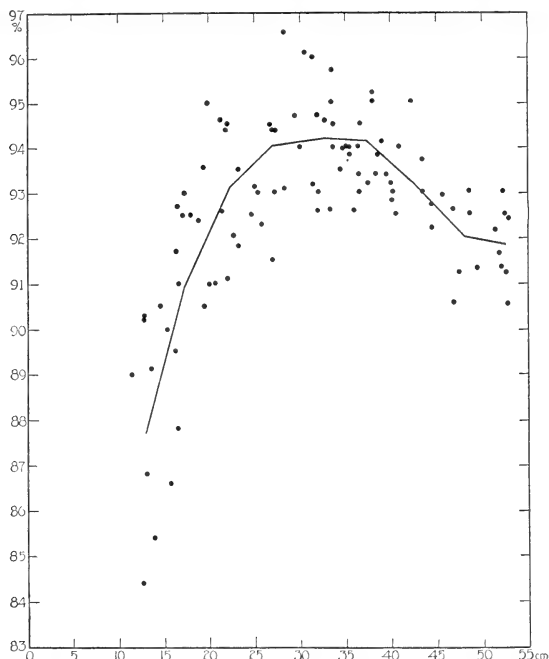


Fig. 7 Field graph and curve (connecting each 5-cm. crown-heel interval) of the per cent which the volume of both cerebral hemispheres forms of the encephalon volume. Abscissa: total body length in cm. Ordinate: hemisphere volume in per cent of the encephalon volume. Individual cases indicated by solid dots. (Data from table 40.)

fronto-occipital diameter is a very constant measure, due perhaps to its close conformance to the skull and to the fact that it is not affected greatly by the torsion of the brain parts or of the brain axis during growth. Its reliability has justified its use to some extent as a basic measurement.

The absolute fronto-occipital diameter (fig. 8) when plotted to the crown-heel length is a straight line ascending from 2 cm. at 10 cm. (C H) to 9 cm. at 50 cm. (C H). Its empirical formula is:

$$\text{Fronto-occipital diameter (cm.)} = 0.175 \text{ CH body length (cm.)} + 0.25$$

TABLE 13

Fronto-occipital diameter

Formula: Fronto-occipital diameter (cm.) = .175 crown-heel length (cm.) + .25
(151 cases)

CROWN-HEEL LENGTH		OBSERVED FRONTO- OCCIPITAL DIAMETER			CALCULATED FRONTO- OCCIPITAL DIAMETER	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maxi- mum	Mini- mum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5	4.0	1.1	0.7	0.9	0.9	0	3
5 to 10	7.1	2.0	1.2	1.6	1.5	-0.1	10
10 to 15	12.3	2.7	1.4	2.3	2.4	+0.1	21
15 to 20	16.7	3.3	2.3	3.2	3.2	0	15
20 to 25	22.6	4.9	3.6	4.3	4.2	-0.1	21
25 to 30	27.0	6.5	4.4	5.1	5.0	-0.1	16
30 to 35	32.6	7.0	5.1	6.0	6.0	0	19
35 to 40	37.1	7.7	6.3	6.8	6.7	-0.1	19
40 to 45	42.3	8.4	7.2	7.8	7.6	-0.2	11
45 to 50	48.2	9.3	7.5	8.6	8.7	+0.1	8
50 to 55	52.2	10.0	8.0	9.2	9.4	+0.2	8

TABLE 14

Calculated fronto-occipital diameter at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	FRONTO-OCCIPITAL DIAMETER	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
cm.	cm.			per cent
5	1.125			12.5
10	2.000	0.875	78.0	22.2
15	2.875	0.875	43.7	31.9
20	3.750	0.875	30.3	41.6
25	4.625	0.875	23.3	51.4
30	5.500	0.875	18.9	61.1
35	6.375	0.875	15.9	70.8
40	7.250	0.875	13.6	80.5
45	8.125	0.875	12.1	90.3
50	9.000	0.875	10.8	100.0
55	9.875	0.875	9.7	109.8

The absolute fronto-occipital diameter when considered in relation to age (fig. 32, curve I) is about 1.3 cm. in the middle of the third fetal month and increases at first rapidly and then more slowly to 9 cm. at birth.

The percentage increment of the fronto-occipital length for 5-cm. intervals of body length (fig. 25, curve I) gradually declines

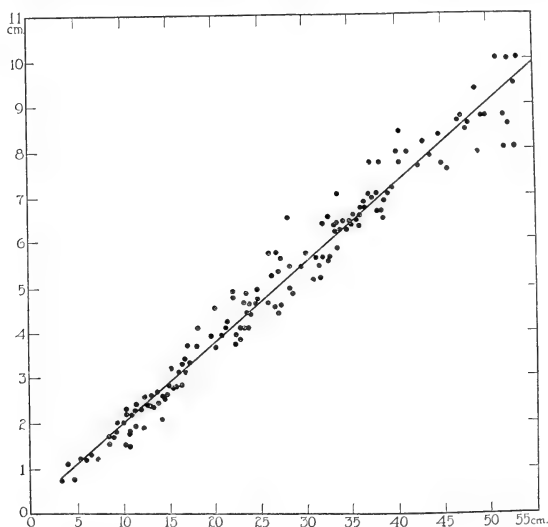


Fig. 8 Field graph and curve of the growth of the cerebral hemispheres in fetal life as shown by the fronto-occipital diameter. Abseissa: total body length in cm. Ordinate: fronto-occipital diameter in cm. Individual cases indicated by solid dots. Curve drawn to the formula. $Y = 0.175X + 0.25$. (Data from tables 13 and 14.)

from about 80 per cent in the 5-to-10-cm. interval to about 10 per cent in the 50-to-55-cm. interval.

The percentage increment of the fronto-occipital length, when calculated for fetal months (fig. 27, curve I), shows an approximate rate of growth of 150 per cent in the third fetal month. This descends rapidly to 31 per cent in the fifth month and gradually to 9.5 per cent in the last fetal month.

The linear measurement from the frontal to the occipital pole indicates therefore a steady absolute cerebral growth in the anteroposterior axis (when compared to crown-heel length) and a constant diminution of the rate of growth (when compared to time).

TABLE 15

Temporal diameter

Formula: Temporal diameter (cm.) = 0.138 crown-heel length (cm.) + 0.3
(141 cases)

CROWN-HEEL LENGTH		OBSERVED TEMPORAL DIAMETER			CALCULATED TEMPORAL DIAMETER	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5	3.9	1.0	0.7	0.85	0.85	0	4
5 to 10	8.1	2.0	1.1	1.45	1.4	-0.03	7
10 to 15	12.4	3.5	1.5	2.1	2.0	-0.1	19
15 to 20	16.6	3.4	1.8	2.6	2.6	0	15
20 to 25	22.6	4.2	2.6	3.3	3.4	+0.1	21
25 to 30	27.0	4.6	3.2	4.1	4.0	-0.1	15
30 to 35	32.6	5.0	3.8	4.5	4.8	+0.3	16
35 to 40	37.1	6.8	4.6	5.5	5.4	-0.1	18
40 to 45	42.3	6.7	5.6	6.2	6.1	-0.1	11
45 to 50	48.2	7.2	6.6	6.7	6.95	+0.25	8
50 to 55	52.2	10.4	6.9	7.9	7.5	-0.4	7

TABLE 16

Calculated temporal diameter at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	TEMPORAL DIAMETER	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
5	0.99			13.7
10	1.68	0.69	69.7	23.4
15	2.37	0.69	41.1	32.9
20	3.06	0.69	29.1	42.6
25	3.75	0.69	22.5	52.1
30	4.44	0.69	18.4	61.6
35	5.13	0.69	15.5	71.3
40	5.82	0.69	13.4	81.0
45	6.51	0.69	11.8	90.4
50	7.20	0.69	10.6	100.0
55	7.89	0.69	9.6	109.5

The curve of absolute temporal diameter (fig. 9) is also a straight line when based on crown-heel length. Starting at about 1 cm. at the 5 cm. (C H), it increases steadily to about 7.2 cm. at 50 cm. (C H). Its empirical formula is:

$$\text{Temporal diameter (cm.)} = 0.138 \text{ C H body length (cm.)} + 0.3$$

When studied in relation to time (fig. 32, curve II), this diameter is found to be 1 cm. at the beginning of the third fetal

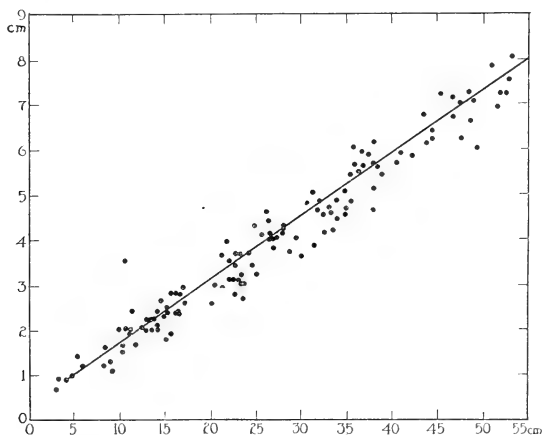


Fig. 9 Field graph and curve of the growth of the cerebral hemispheres in fetal life as shown by the temporal diameter. Abcissa: total body length in cm. Ordinate: temporal diameter in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = 0.138X + 0.3$. (Data from tables 15 and 16.)

month and to rise at first fairly rapidly and then more slowly to 7.2 cm. at birth.

The rate of growth of the temporal diameter (fig. 25, curve II) decreases steadily from 70 per cent for the 5-to-10-cm. interval to about 10 per cent for the 50-to-55-cm. interval.

The percentage increment of the temporal diameter, when calculated for fetal months (fig. 27, curve II), is approximately 70 per cent in the fourth fetal month and falls to about 35 per

cent in the fifth month and to 9.2 per cent in the tenth month. Or, in other words, the transverse axis of the cerebral hemispheres shows a constant diminution in the rate of growth throughout the fetal period.

TABLE 17

Volume of the cerebellum

Formula: Volume of cerebellum (cc.) = .01 (.095 crown-heel length
(cm.))^{4.3} + 20.0
(114 cases)

CROWN-HEEL LENGTH		OBSERVED VOLUME OF CEREBELLUM			CALCULATED VOLUME OF CEREBELLUM	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5							
5 to 10							
10 to 15	12.8	0.5	0.1	0.3	0.5	+0.2	10
15 to 20	16.9	0.7	0.2	0.4	0.5	+0.1	14
20 to 25	22.6	2.0	0.5	0.9	0.6	-0.3	18
25 to 30	27.0	2.7	0.9	1.3	1.2	-0.1	13
30 to 35	32.7	4.0	1.0	2.4	2.8	+0.4	16
35 to 40	37.2	9.3	3.4	5.4	5.1	-0.3	17
40 to 45	42.3	11.5	5.7	8.9	9.3	+0.4	11
45 to 50	48.0	22.0	14.0	19.0	17.1	-2.0	7
50 to 55	52.2	26.0	18.0	21.3	25.7	+4.4	8

TABLE 18

Calculated cerebellum volume at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH		CEREBELLUM VOLUME		INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN	
cm.		cc.		cc.	per cent	per cent	
5							
10		0.20					1.0
15		0.28		0.08	40.0		1.3
20		0.43		0.15	53.6		2.1
25		0.89		0.46	107.0		4.3
30		1.89		1.00	112.0		9.1
35		3.80		1.91	100.1		18.2
40		7.13		3.33	87.8		34.1
45		12.60		5.47	76.8		60.3
50		20.90		8.30	65.8		100.0
55		33.40		12.50	59.8		160.0

Growth of the cerebellum

The growth of the cerebellum is portrayed by the graphs in figures 10 to 13, inclusive, and by the outline sketches in figure 38. The absolute volume of the cerebellum, when plotted against

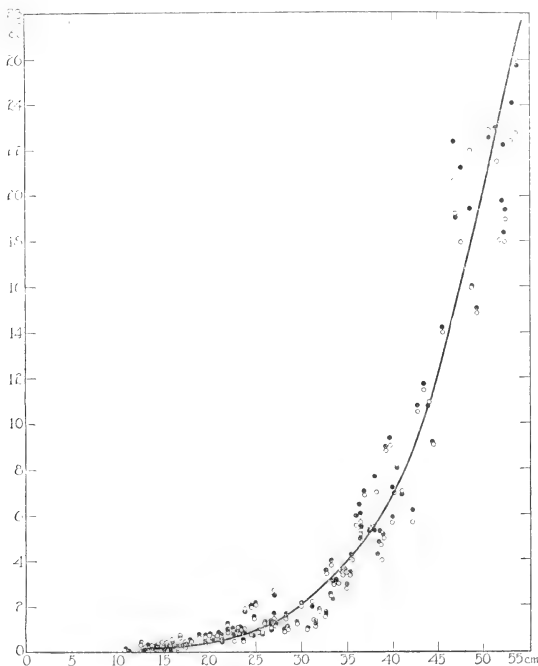


Fig. 10 Field graph and curve of the growth of the cerebellum in weight and volume in fetal life. Abscissa: total body length in cm. Ordinate: cerebellum weight and volume in grams (cc.). Individual cases indicated by solid dots (for weight) and by circles (for volume). Curve drawn to the formula: $Y = 0.01 [(0.095X)^{4.9} + 20.0]$. (Data from tables 17 and 18.)

crown-heel body length (fig. 10), presents a concave volume curve expressed by the empirical formula:

$$\text{Volume of the cerebellum (cc.)} = 0.01 (0.095 \text{ C H body length (cm.)})^{4.9} + 20.0$$

The cerebellum is not grossly visible before the fetus is about 10 cm. (C H) long. At 20 cm. (C H) its volume is about 0.4 cc., and this increases slowly to about 2 cc. at 30 cm. (C H). The

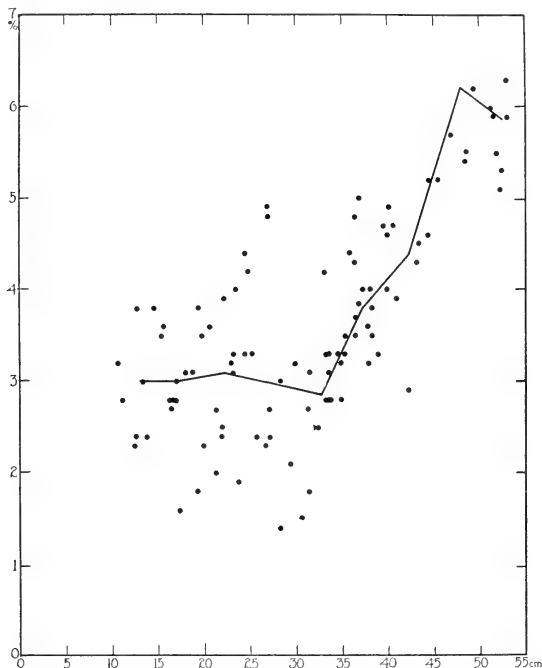


Fig. 11 Field graph and curve (connecting the average points of each 5-cm. crown-heel interval) of the per cent which the volume of the cerebellum forms of the encephalon volume. Abscissa: total body length in cm. Ordinate: cerebellum volume in per cent of entire brain volume. Individual cases indicated by solid dots. (Data from table 40.)

absolute volume curve then turns sharply upward and rises very rapidly to 21 cc. at 50 cm. (C H).

On the basis of time (figs. 34 and 35), the volume of the cerebellum is about 0.4 cc. at four fetal months and approximately

2.3 cc. at six fetal months. At this time it begins to enlarge rapidly, increasing almost ten-fold in the last four months of intra-uterine life and reaching a volume of 21 cc. at birth. This tremendous growth in volume in the last three or four months of fetal life, at a time when all other brain parts are growing far less rapidly, is a peculiar characteristic of the cerebellum.

The percentage increment of the cerebellum volume (fig. 26, curve VII) reflects the peculiarities of the curve of absolute volume. Starting at a comparatively low value, it rises to a peak of 112 per cent for the 25-to-30-cm. interval and descends to 60 per cent in the 50-to-55-cm. interval.

The percentage increment of the cerebellum volume, when calculated for time (fig. 28, curve IV), shows an approximate rate of growth of about 160 per cent in the fifth month, and then falls rapidly to 54 per cent for the tenth month.

The relative growth of the cerebellum is also illustrated by the per cent which it forms of the entire brain volume at various periods in fetal life (fig. 11). This value is, with slight variations, about 3 per cent until the middle of the sixth fetal month. At this time the tremendous growth of the cerebellum becomes an important factor in the total increase of the brain as a whole, and by birth it forms about 6 per cent of the entire brain volume.

The absolute length of the vermis cerebelli, when plotted to crown-heel body length (fig. 12), is expressed by the formula:

$$\text{Vermis cerebelli length (cm.)} = 0.01 [(\text{C H length (cm.)})^{1.41} + 0.15]$$

It also portrays the great growth of this part of the brain in the last four fetal months. At 10 cm. (C H) the vermis cerebelli length is only about 0.4 cm. It ascends in a very shallow curve to 2.6 cm. at 50 cm. (C H).

The absolute length of the vermis cerebelli when calculated for fetal months (fig. 31, curve I) rises in practically a straight line from 0.4 cm. at the beginning of the fourth month to 2.64 cm. at birth.

The rate of growth of the vermis cerebelli length (fig. 25, curve III) shows a gradual decrease from 50 per cent in the 10-to-15-cm. interval to 13 per cent at the 50-to-55-cm. interval. The entire

percentage increment curve, however, is much higher than any other percentage increment based upon a straight-line measurement (with the single exception of the vermis cerebelli height, fig. 25, curve IV).

TABLE 19

Length of the vermis cerebelli

Formula: Vermis cerebelli length (cm.) = .01 [(crown-heel length (cm.))^{1.41} + 0.15]
(124 cases)

CROWN-HEEL LENGTH		LENGTH OF VERMIS CEREBELLI			CALCULATED LENGTH OF VERMIS CEREBELLI	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5							
5 to 10							
10 to 15	12.7	0.9	0.3	0.5	0.5	0.0	16
15 to 20	16.9	1.1	0.4	0.6	0.7	+0.1	14
20 to 25	22.5	1.2	0.6	0.9	1.0	+0.1	18
25 to 30	27.0	1.8	0.9	1.15	1.2	+0.05	14
30 to 35	32.8	1.7	1.1	1.5	1.5	0	16
35 to 40	37.1	2.6	1.3	1.8	1.8	0	18
40 to 45	42.2	2.5	1.7	2.1	2.1	0	12
45 to 50	48.2	2.7	2.2	2.5	2.5	0	8
50 to 55	52.2	3.4	2.5	2.7	2.8	+0.1	8

TABLE 20

Calculated vermis cerebelli length at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	VERMIS CEREBELLI LENGTH	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
cm.	cm.			per cent
5				
10	0.41			15.5
15	0.61	0.20	49.0	23.1
20	0.83	0.22	36.1	31.5
25	1.08	0.25	30.2	40.9
30	1.36	0.28	25.9	51.6
35	1.65	0.29	21.3	62.6
40	1.96	0.31	18.8	74.3
45	2.29	0.33	16.8	86.8
50	2.64	0.35	15.3	100.0
55	2.99	0.35	13.3	113.2

The percentage increment of the vermis cerebelli length when calculated for fetal months (fig. 27, curve VIII) is approximately 85 per cent in the fifth month, descending at first swiftly and then more slowly to about 12.5 per cent in the tenth month.

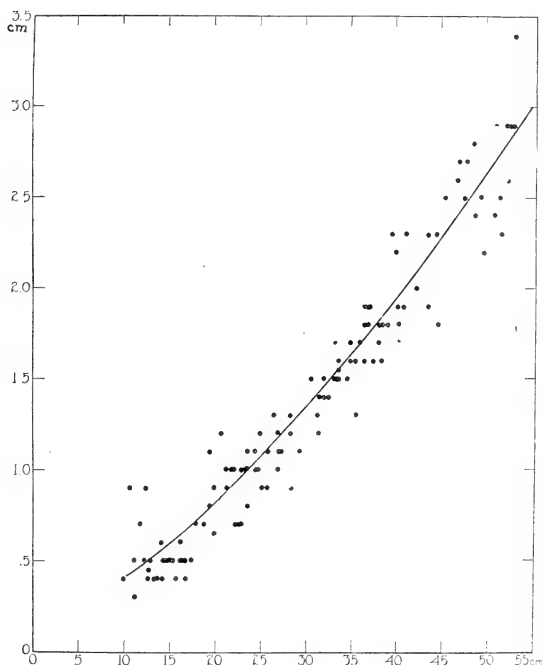


Fig. 12 Field graph and curve of the growth of the cerebellum in fetal life as shown by the vermis cerebelli length. Abscissa: total body length in cm. Ordinate: vermis cerebelli length in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = 0.01 (X^{1.41} + 0.15)$. (Data from tables 19 and 20.)

The absolute height of the vermis cerebelli when plotted against crown-heel body length (fig. 13) also forms a shallow curve, expressed empirically by the formula:

$$\text{Vermis cerebelli height (cm.)} = 0.01 (\text{C H body length (cm.)})^{1.37}$$

It is 0.23 cm. at 10 cm. (C H) and rises to about 2.1 cm. at 50 cm. (C H).

TABLE 21

Height of the vermis cerebelli

Formula: Vermis cerebelli height (cm.) = .01 (crown-heel length (cm.))^{1.37}
(122 cases)

CROWN-HEEL LENGTH		OBSERVED HEIGHT OF VERMIS CEREBELLI			CALCULATED HEIGHT OF VERMIS CEREBELLI	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5							
5 to 10							
10 to 15	12.7	0.6	0.3	0.4	0.3	-0.1	14
15 to 20	16.9	0.7	0.2	0.5	0.5	0	14
20 to 25	22.6	0.9	0.5	0.7	0.7	0	18
25 to 30	27.0	1.0	0.6	0.8	0.9	+0.1	14
30 to 35	32.8	1.4	0.8	1.1	1.2	+0.1	16
35 to 40	37.1	1.9	1.0	1.4	1.4	0	18
40 to 45	42.2	2.0	1.1	1.6	1.7	+0.1	12
45 to 50	48.2	2.5	1.6	2.0	2.0	0	8
50 to 55	52.2	2.8	1.6	2.2	2.2	0	8

TABLE 22

Calculated vermis cerebelli height at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	VERMIS CEREBELLI HEIGHT	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
cm.	cm.			per cent
5				
10	0.23			10.8
15	0.41	0.18	78.1	19.2
20	0.61	0.20	48.8	26.6
25	0.82	0.21	34.4	38.5
30	1.06	0.24	29.2	49.8
35	1.31	0.25	23.6	61.5
40	1.57	0.26	19.8	73.7
45	1.84	0.27	17.2	86.3
50	2.13	0.29	15.8	100.0
55	2.42	0.29	12.0	113.7

The absolute height of the vermis cerebelli when calculated for age in fetal months (fig. 31, curve II) rises in practically a straight line from 0.23 cm. at the beginning of the fourth month to 2.1 cm. at birth.

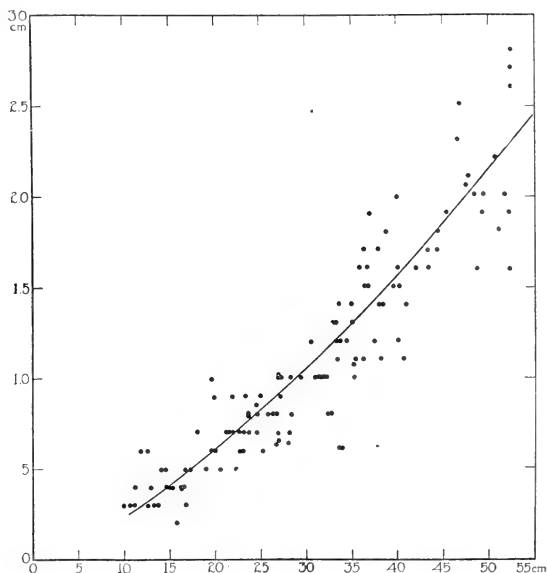


Fig. 13 Field graph and curve of the growth of the cerebellum in fetal life as shown by the vermis cerebelli height. Abscissa: total body length in cm. Ordinate: vermis cerebelli height in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = 0.01 (X^{1.37})$. (Data from tables 21 and 22.)

The percentage increment of the vermis cerebelli height when calculated for 5-cm. intervals of crown-heel length (fig. 25, curve IV) is similar to that of the vermis cerebelli length except that it is somewhat greater at first and falls more rapidly.

The percentage increment of the vermis cerebelli height, when calculated for fetal months (fig. 27, curve IX), shows a rate of

growth of about 126 per cent for the fourth month, falls to 58 per cent for the fifth month, and then descends steadily to about 13 per cent for the tenth month.

The growth of the cerebellum during the latter part of fetal life is also strikingly illustrated by the midsagittal sections shown in figures 37 and 38. In the third fetal month (figure 38, C) the cerebellum grows posteriorly and parallel to the pons and medulla; by the middle of the sixth fetal month (fig. 38, D, E, F) it assumes its characteristic shape and position in relation to the cerebral hemispheres. From the middle of the sixth fetal month to birth (fig. 38, G to I, inclusive) each succeeding tracing indicates a greater relative size in this portion of the brain.

It will be noted in this connection that the graphs of absolute growth of the cerebellum in both of the linear dimensions resemble volume curves much more closely than does the graphic expression of any other lineal dimensions of the nervous system.

Growth of the pons and medulla

The growth of the pons and medulla (fig. 14) presents a typical volume curve having the empirical formula:

$$\text{Pons and medulla volume (cc.)} = 0.01 [(0.2 \text{ C H body length (cm.)})^{2.67} + 20.0]$$

At 10 cm. (C H) their volume is about 0.3 cc., by 30 cm. (C H) it is 1.4 cc., and at birth (50 cm. C H) it is approximately 5 cc. When calculated for age in fetal months (fig. 35, curve II), their volume is 0.2 cc. at three months and 1.4 cc. at six months. From this time it rises steadily to about 5 cc. at birth.

The percentage increment of the volume (fig. 26, curve VIII) is approximately 50 per cent for the 10-to-15-cm. (C H) interval and rises to about 56 to 57 per cent for the interval between 15 to 20 cm. It then descends steadily to about 27 per cent from 50 to 55 cm. (C H).

The percentage increment of the pons and medulla volume as calculated for fetal months (fig. 28, curve II) shows a rate of growth of about 96 per cent for the fourth month, and then descends steadily to about 27.5 per cent for the last month of prenatal life.

One of the most interesting facts concerning the prenatal growth of the pons and medulla is the per cent of the entire brain volume which they form during fetal life (fig. 15 and table 40). In the middle of the fourth fetal month the pons and medulla make up 5.5 per cent of the entire brain volume, at 5 months they

TABLE 23

Volume of the pons and medulla

Formula: Pons and medulla volume (cc.) = .01 [(2 crown-heel length (cm.))^{2.67} + 20]
(112 cases)

CROWN-HEEL LENGTH		OBSERVED PONS AND MEDULLA VOLUME			CALCULATED PONS AND MEDULLA VOLUME	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5							
5 to 10							
10 to 15	12.6	0.5	0.2	0.3	0.3	0	11
15 to 20	16.9	0.7	0.15	0.4	0.5	+0.1	14
20 to 25	22.5	1.0	0.4	0.8	0.8	0	16
25 to 30	27.0	1.4	0.5	0.9	1.1	+0.2	13
30 to 35	32.7	2.1	0.9	1.5	1.7	+0.2	17
35 to 40	37.0	3.2	1.6	2.4	2.3	-0.1	16
40 to 45	42.3	3.9	2.5	3.1	3.2	+0.1	11
45 to 50	48.0	5.5	3.7	4.3	4.4	+0.1	6
50 to 55	52.2	6.3	4.5	5.2	5.4	+0.2	8

TABLE 24

Calculated pons and medulla volume at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	PONS AND MEDULLA VOLUME	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cc.	per cent	
cm.	cc.			per cent
5				
10	0.26			5.3
15	0.39	0.13	50.0	8.0
20	0.61	0.22	56.5	12.5
25	0.94	0.33	54.1	19.3
30	1.40	0.46	49.0	28.7
35	2.01	0.61	43.5	41.2
40	2.78	0.77	38.3	57.0
45	3.73	0.95	34.2	76.4
50	4.88	1.15	30.8	100.0
55	6.23	1.35	27.7	128.0

form only about 2.7 per cent, and from that time on to birth they gradually decrease in relative volume, so that they form only 1.5 per cent of the entire brain volume in the new-born. This

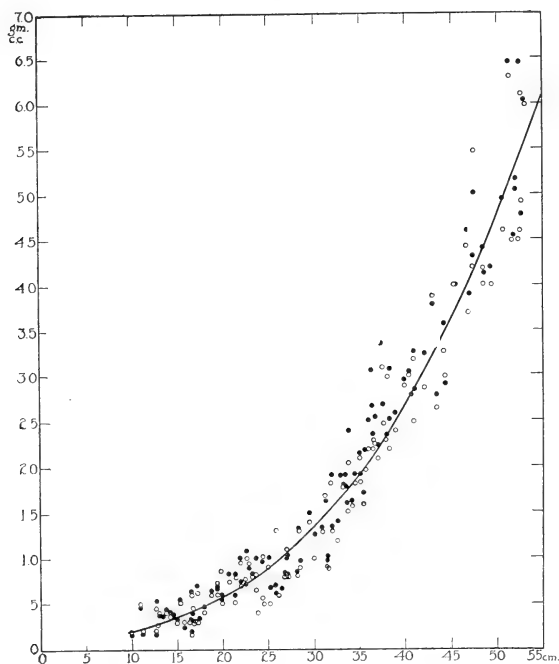


Fig. 14 Field graph and curve of the growth of the pons and medulla in weight and volume in fetal life. Abscissa: total body length in cm. Ordinate: pons and medulla weight and volume in grams (cc.). Individual cases indicated by solid dots (for weight) and by circles (for volume). Curve drawn to the formula: $Y = 0.01 [(0.2X)^{2.67} + 20.0]$. (Data from tables 23 and 24.)

relative decline is clearly due to the great increase in the absolute volume of the cerebral hemispheres and of the cerebellum during the last half of fetal life.

The pons length also shows some interesting changes in the course of fetal life. The graph of absolute pons length plotted against total body length (fig. 16) is practically a straight line having the formula:

Length of pons (cm.) = 0.0263 C H body length (cm.) + 0.16

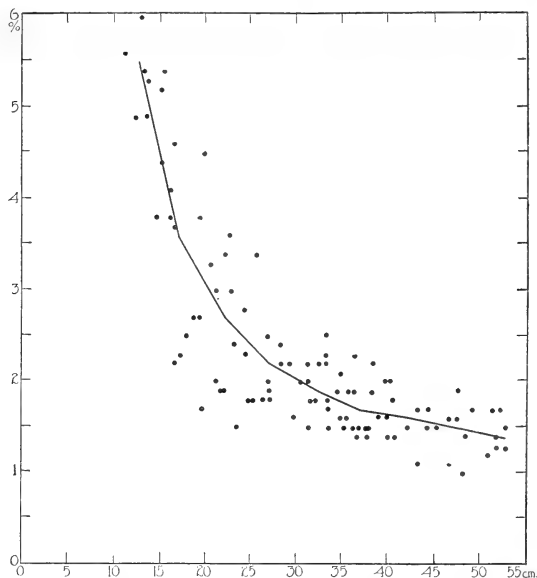


Fig. 15 Field graph and curve (connecting the average points of each 5-cm. crown-heel interval) of the per cent which the volume of the pons and medulla forms of the encephalon volume. Abseissa: total body length in cm. Ordinate: pons and medulla volume in per cent of the entire brain volume. Individual cases indicated by solid dots. (Data from table 40.)

The length increases steadily from 0.42 cm. at 10 cm. (C H) to about 1.53 cm. at birth (50 cm. C H).

When plotted against time, the pons length increases rather rapidly from the third to the beginning of the sixth month, and then grows more slowly at a uniform rate until birth.

The percentage increment of the pons length against intervals of growth in body length (fig. 25, curve V) starts at 30 per cent between 10 and 15 cm. (C H) and falls steadily but slowly to 9.8 per cent in the 50-to-55-cm. interval.

The percentage increment of the pons length as calculated for fetal months (fig. 27, curve III) shows a rate of growth of about

TABLE 25

Pons length

Formula: Pons length (cm.) = 0.0263 crown-heel length (cm.) + 0.16
(124 cases)

CROWN-HEEL LENGTH		OBSERVED PONS LENGTH			CALCULATED PONS LENGTH	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	
0 to 5							
5 to 10							
10 to 15	12.2	0.6	0.3	0.5	0.5	0	18
15 to 20	16.7	0.8	0.5	0.6	0.6	0	13
20 to 25	22.6	0.9	0.7	0.8	0.8	0	18
25 to 30	27.0	1.1	0.6	0.9	0.9	0	13
30 to 35	32.8	1.2	0.9	1.0	1.0	0	16
35 to 40	37.1	1.5	1.0	1.2	1.1	-0.1	18
40 to 45	42.2	1.4	1.0	1.3	1.3	0	12
45 to 50	48.2	1.6	1.2	1.35	1.4	-0.05	8
50 to 55	52.2	1.7	1.3	1.5	1.5	0	8

TABLE 26

Calculated pons length at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	PONS LENGTH	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		<i>cm.</i>	<i>per cent</i>	
<i>cm.</i>	<i>cm.</i>			<i>per cent</i>
5	0.29			
10	0.42	0.13+	44.8	28.7
15	0.55	0.13+	30.0	37.6
20	0.68	0.13+	24.8	46.5
25	0.81	0.13+	19.2	55.4
30	0.94	0.13+	16.1	64.3
35	1.08	0.13+	13.9	73.3
40	1.21	0.13+	12.2	82.3
45	1.34	0.13+	11.8	91.2
50	1.47	0.13+	9.8	100.0
55	1.60	0.13+	8.9	108.8

75 per cent in the third month and descends steadily to approximately 7 per cent in the tenth month.

There is no doubt that the pons and medulla form a large part of the brain in early fetal life. Figures 38, a, b, c, and d, substantiate the percentage curve (fig. 18). They show that the pons and medulla form a very large portion of the entire brain volume at this time. By the middle of the third fetal month (fig. 38, b) fully one-third of the brain, as it is seen in mid-

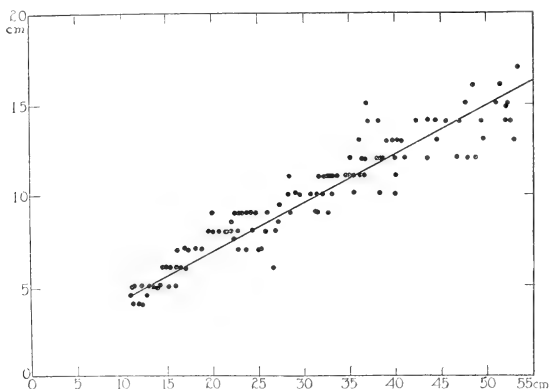


Fig. 16 Field graph and curve of the growth of the pons and medulla in fetal life, as shown by the pons length. Abscissa: total body length in cm. Ordinate: pons length in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = 0.0263X + 0.16$. (Data from tables 25 and 26.)

sagittal section, is formed by the pons and medulla. In the fourth and fifth months (figs. 38, b, c, d, and e) the pons and medulla become relatively smaller.

Growth of the midbrain

A consideration of the colliculi and the midbrain does not lead to quite such clean-cut results as does the study of the other brain parts. The midbrain, as it was prepared for the volume and weight determinations, included not only the colliculi above the iter, but also a portion of the brain stem below it.

The absolute volume of the midbrain when plotted against body length (fig. 17) presents a shallow curve and has the empirical formula:

$$\text{Volume of midbrain (cc.)} = 0.01 [(0.168 \text{ C H length (cm.)})^{2.56} + 12.0]$$

TABLE 27

Volume of the midbrain

Formula: Midbrain volume (cc.) = .01 [(.168 crown-heel length (cm.))^{2.56} + 12]
(109 cases)

CROWN-HEEL LENGTH		OBSERVED MIDBRAIN VOLUME			CALCULATED VOLUME OF MIDBRAIN	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maxi- mum	Mini- mum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5							
5 to 10							
10 to 15	12.9	0.3	0.1	0.2	0.2	0	9
15 to 20	16.9	0.4	0.2	0.3	0.3	0	14
20 to 25	22.5	0.8	0.3	0.5	0.4	-0.1	17
25 to 30	27.0	0.9	0.4	0.6	0.6	0	13
30 to 35	32.7	1.4	0.4	0.8	0.9	+0.1	16
35 to 40	37.3	1.7	0.8	1.4	1.2	-0.2	14
40 to 45	42.3	2.2	1.2	1.6	1.6	0	11
45 to 50	48.0	3.1	1.5	2.4	2.2	-0.2	7
50 to 55	52.2	3.0	1.8	2.4	2.7	+0.3	8

TABLE 28

Calculated midbrain volume at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH		MIDBRAIN VOLUME		INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
cm.		cc.		cc.	per cent	
5						
10		0.16				6.6
15		0.23		0.07	43.7	9.4
20		0.34		0.11	47.8	13.9
25		0.51		0.17	50.0	20.9
30		0.75		0.24	47.0	30.7
35		1.05		0.30	40.0	43.0
40		1.43		0.38	36.2	58.5
45		1.89		0.46	32.0	77.4
50		2.44		0.55	29.1	100.0
55		3.12		0.68	27.8	128.0

At 13 cm. (C H) the absolute midbrain volume is about 0.2 cc., and this steadily rises to 0.75 cc. at 30 cm. (C H). From this point the absolute volume increases at a faster pace to about 2.5 cc. at 50 cm. (C H).

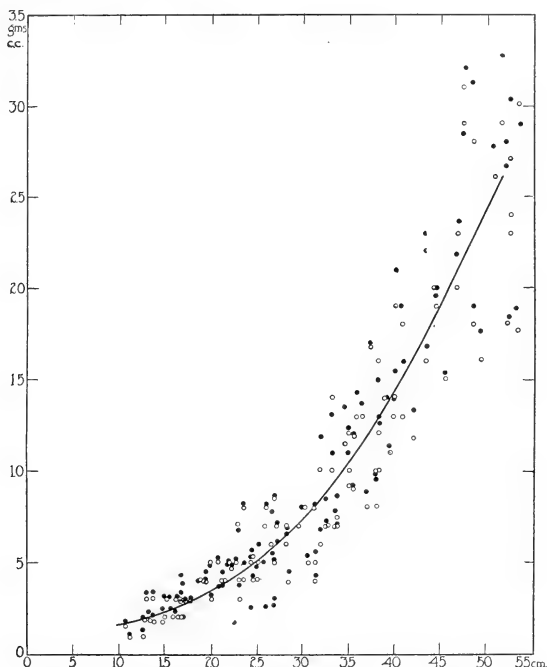


Fig. 17 Field graph and curve of the growth of the midbrain in weight and volume in fetal life. Abscissa: total body length in cm. Ordinate: midbrain weight and volume in grams (cc.). Individual cases indicated by solid dots (for weight) and by circles (for volume). Curve drawn to the formula: $Y = 0.01 [(0.168X)^{2.56} + 12.0]$. (Data from tables 27 and 28.)

The volume of the midbrain, when calculated for age in fetal months (fig. 35, curve III), is about 0.15 cc. at the third month and increases to about 0.8 cc. at six months. Thereafter it grows slowly to about 2.5 cc. at birth.

The percentage increment of the midbrain volume, when calculated for 5-cm. intervals of crown-heel length (fig. 26, curve IX), is about 45 per cent for the 10-to-15-cm. interval. It rises slightly at the 20-to-25-cm. interval and descends to 30 per cent for the 50-to-55-cm. interval.

The percentage increment of the midbrain volume as calculated for fetal months (fig. 28, curve III) is approximately 81 per cent

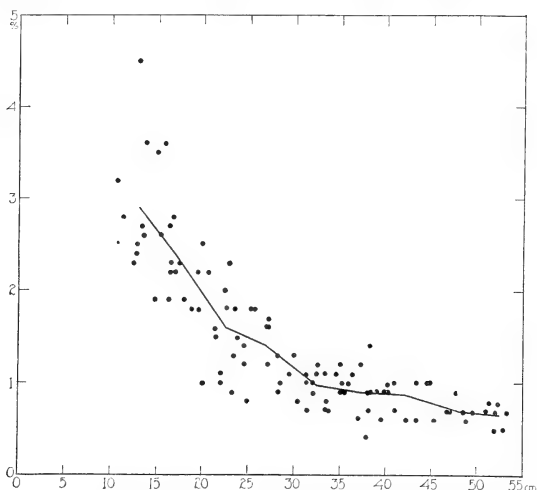


Fig. 18 Field graph and curve (connecting the average points of each 5-cm. crown-heel interval) of the per cent which the midbrain volume forms of the encephalon volume. Abscissa: total body length in cm. Ordinate: mid-brain volume in per cent of the encephalon volume. Individual cases indicated by solid dots. (Data from table 40.)

in the fourth month and descends to about 25 per cent in the last fetal month.

The slow growth of the midbrain is reflected in the per cent which this brain part forms of the entire brain volume (figure 18 and table 40). Beginning at 2.9 per cent at three months, it steadily declines to but 0.7 per cent of the entire brain volume at birth.

The length of the colliculi is not a true index of the mid-brain growth in early fetal life, and its significance as a measure of growth of that portion of the mesencephalon below the iter is particularly questionable. In some fetuses of three to four months a sharp line of demarcation between the superior colliculi and the thalamencephalon may be present, while in other specimens of the same age no line of separation can be distinguished.

This conclusion is supported by evidence shown in figures 36, a, b, c. Figures 36, a, and 36, b, of specimens prior to the third fetal month show no colliculi; figure 36, c, shows colliculi well marked off and relatively larger than in a new-born specimen.

The curve of the absolute length of the colliculi, when plotted against body length (fig. 19), is a straight line. It starts at 0.7 cm. at 15 cm. (C H) and rises to 1.22 cm. in length at 50 cm. (C H). It may be expressed by the empirical formula:

$$\text{Length of colliculi (cm.)} = 0.015 \text{ C H body length (cm.)} \\ + 0.47$$

The growth of the colliculi in absolute length when plotted against time (fig. 33, curve VI) approaches a straight line, although the rate of growth is more rapid in the fourth and fifth months than in the last four fetal months.

Growth of the spinal cord

The growth of the spinal cord resembles that of the brain and particularly that of the pons and medulla. The absolute volume of the spinal cord plotted against crown-heel length (fig. 21) is a very shallow but typical volume curve, which may be expressed by the empirical formula:

$$\text{Volume of the spinal cord (cc.)} = 0.01 [(0.17 \text{ C H} \\ \text{length (cm.)})^{2.57} + 11.0]$$

The absolute volume at 15 cm. (C H) is approximately 0.22 cc. The curve rises steadily until it reaches about 1 cc. at 35 cm. (CH). From this point it proceeds upward at a sharper pitch, reaching about 2.5 cc. at 50 cm. (C H).

The growth in absolute volume against time (fig. 35, curve IV) corresponds almost exactly with that of the midbrain and shows

a fairly rapid rate of absolute growth from the third to the beginning of the fifth month and a slower and more regular growth thereafter.

The data of Jackson ('09) (table 35) show a uniformly higher average for the absolute spinal cord volumes. His small fetuses

TABLE 29

Length of the colliculi

Formula: Colliculi length (cm.) = .015 crown-heel length (cm.) + .47.

(117 cases)

CROWN-HEEL LENGTH		OBSERVED LENGTH OF THE COLLICULI			CALCULATED LENGTH OF THE COLLICULI	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5							
5 to 10							
10 to 15	13.0	0.9	0.5	0.66	0.66	0	11
15 to 20	16.9	0.9	0.5	0.74	0.73	-0.01	14
20 to 25	22.5	1.0	0.7	0.80	0.81	+0.01	18
25 to 30	26.9	1.0	0.7	0.90	0.87	-0.03	12
30 to 35	32.8	1.7	0.8	0.99	0.96	-0.03	16
35 to 40	37.1	1.1	0.9	0.98	1.03	+0.05	18
40 to 45	42.2	1.2	1.0	1.10	1.10	0	12
45 to 50	48.3	1.4	1.1	1.23	1.20	-0.03	8
50 to 55	52.2	1.3	1.1	1.20	1.25	+0.05	8

TABLE 30

Calculated length of the colliculi at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	LENGTH OF COLLICULI	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
cm.	cm.			per cent
5				
10				
15	0.695			57.0
20	0.775	0.75	10.8	63.1
25	0.845	0.75	9.8	69.3
30	0.920	0.75	8.9	75.4
35	0.995	0.75	8.2	81.5
40	1.070	0.75	7.6	87.8
45	1.145	0.75	7.0	93.9
50	1.220	0.75	6.6	100.0
55	1.295	0.75	6.2	106.2

(obtained by sectioning) show an absolute volume of 0.034 cc. at approximately the close of the first month, while his two largest fetuses (new-born) have a cord volume of about 3 cc.

The percentage increment of the spinal cord volume when determined for 5-cm. intervals of crown-heel body length (fig. 26,

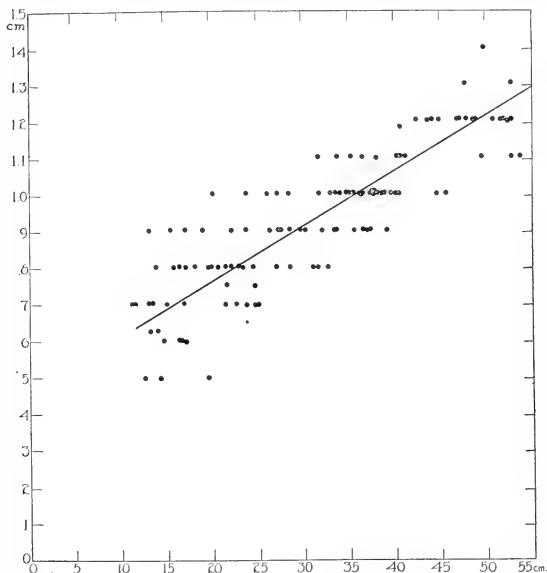


Fig. 19 Field graph and curve of the growth of the midbrain in fetal life as shown by the colliculi length. Abscissa: total body length in cm. Ordinate: colliculi length in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = 0.015X + 0.47$. (Data from tables 29 and 30.)

curve X) is 47 per cent for the 10-to-15-cm. interval. It rises to about 55 per cent for the 15-to-20-cm. interval, and then falls to about 25 per cent for the 50-to-55-cm. interval.

The percentage increment of the spinal cord volume when calculated for fetal months (fig. 28, curve I) shows a rate of growth of approximately 94 per cent in the fourth month and then descends steadily to 26 per cent in the last fetal month.

The relation of the spinal cord volume to the volume of the entire brain is shown in figure 22 and in table 40. At the middle of the second month the cord volume is equal to 4.4 per cent of the total brain volume. It then drops rapidly at first and afterward more slowly to about 0.85 per cent of the total brain volume at birth.

TABLE 31

Frontospinal length

Formula: Frontospinal length (cm.) = (2 crown-heel length (cm.))⁴⁷ - 2.2
(145 cases)

CROWN-HEEL LENGTH		OBSERVED FRONTO- SPINAL LENGTH			CALCULATED FRONTOSPINAL LENGTH	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maxi- mum	Mini- mum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5	4.0	1.0	0.7	0.9	0.5	-0.5	3
5 to 10	6.8	1.7	1.0	1.4	1.2	-0.2	9
10 to 15	12.1	2.9	1.9	2.1	2.3	+0.2	20
15 to 20	16.6	3.5	2.6	2.9	3.0	+0.1	14
20 to 25	22.6	4.5	3.0	3.8	3.8	0	20
25 to 30	27.0	4.6	3.4	4.1	4.3	+0.2	15
30 to 35	32.8	5.3	4.3	4.8	4.9	+0.1	17
35 to 40	37.1	6.5	4.0	5.3	5.4	+0.1	19
40 to 45	42.2	6.8	5.1	5.9	5.8	-0.1	12
45 to 50	48.2	6.9	5.9	6.3	6.4	+0.1	8
50 to 55	52.2	7.3	6.3	6.8	6.7	-0.1	8

TABLE 32

Calculated frontospinal length at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	CALCULATED FRONTO- SPINAL LENGTH	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
5	0.75			11.5
10	1.89	1.14	152.0	29.0
15	2.75	0.86	44.8	42.2
20	3.46	0.71	28.2	53.1
25	4.09	0.63	18.2	62.8
30	4.65	0.56	13.4	71.4
35	5.17	0.52	12.4	79.5
40	5.64	0.47	9.3	86.6
45	6.09	0.45	8.0	94.5
50	6.51	0.42	6.7	100.0
55	6.91	0.40	6.3	106.2

The absolute length of the spinal cord (fig. 23) ascends rather sharply from 2.2 cm. length at 5 cm. (C H) to 9.2 cm. at 25 cm. (C H). It then ascends more slowly to 14.2 cm. at birth (50 cm. C H). Its empirical formula is:

$$\text{Length of spinal cord (cm.)} = (10.0 \text{ C H body length (cm.)})^{.467} - 4.0$$

The curve of cord length, when plotted against time, is quite similar to that plotted against body length, but the rise is a more rapid one (fig. 32, curve IV).

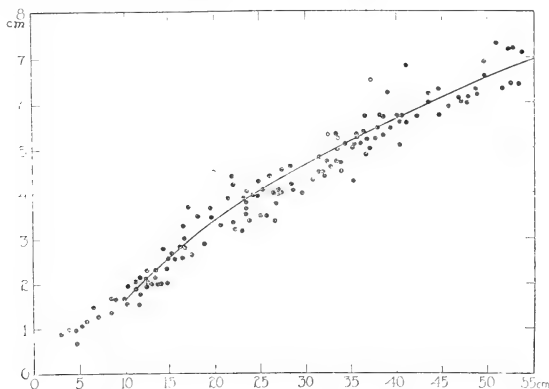


Fig. 20 Field graph and curve of the growth of the brain stem in fetal life as shown by the fronto-spinal length. Abscissa: total body length in cm. Ordinate: fronto-spinal length in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = (2.0X)^{.47} - 2.2$. (Data from tables 31 and 32.)

The percentage increment of the cord length as calculated for 5-cm. intervals of crown-heel length (fig. 25, curve VIII) is about 109 per cent for the 5-to-10-cm. interval, falls rapidly to 23 per cent for the 15-to-20-cm. interval, and then gradually to 6 per cent for the 50-to-55-cm. interval.

The percentage increment of the spinal-cord length when calculated for fetal months (fig. 27, curve V) shows a rate of growth of approximately 400 per cent in the third month, descending rapidly to 26 per cent in the sixth month, and then gradually to nearly 6 per cent in the last month of the fetal period.

DISCUSSION

The discussion of the results of this work will be limited to, 1) a comparison of the growth of the central nervous system as a whole with that of the various other viscera and parts of the body in the fetal period; 2) the analysis of the type of growth of the

TABLE 33

Volume of the spinal cord

Formula: Spinal cord volume (cc.) = .01 [(0.17 crown-heel length (cm.))^{2.57} + 11.]
(128 cases)

CROWN-HEEL LENGTH		OBSERVED VOLUME OF SPINAL CORD			CALCULATED VOLUME OF SPINAL CORD	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cc.	cc.	cc.	cc.	cc.	
0 to 5							
5 to 10	7.2	0.05	0.05	0.05	0.13	0.08	3
10 to 15	12.5	0.3	0.1	0.2	0.2	0	18
15 to 20	16.5	0.5	0.1	0.3	0.3	0	14
20 to 25	22.6	0.7	0.3	0.5	0.4	-0.1	19
25 to 30	26.9	1.1	0.3	0.65	0.6	0	14
30 to 35	32.6	1.4	0.5	0.9	0.9	0	17
35 to 40	37.0	2.1	0.7	1.1	1.1	0	17
40 to 45	41.9	2.3	1.2	1.7	1.7	0	11
45 to 50	48.0	3.1	1.4	2.4	2.3	-0.1	7
50 to 55	52.2	3.8	1.8	2.5	2.8	+0.3	8

TABLE 34

Calculated spinal cord volume at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	SPINAL CORD VOLUME	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cc.	per cent	
cm.	cc.			per cent
5				
10	0.15			5.9
15	0.22	0.07	46.7	8.6
20	0.34	0.12	54.5	13.3
25	0.52	0.18	53.0	20.3
30	0.77	0.25	48.1	30.1
35	1.09	0.32	41.5	42.5
40	1.49	0.40	36.7	58.2
45	1.98	0.49	32.9	77.4
50	2.56	0.58	29.2	100.0
55	3.24	0.68	24.5	126.5

central nervous system into its component parts; 3) observations upon the relation of volumetric and lineal measurements of the nervous system, and, 4) a summary of the proportions which the

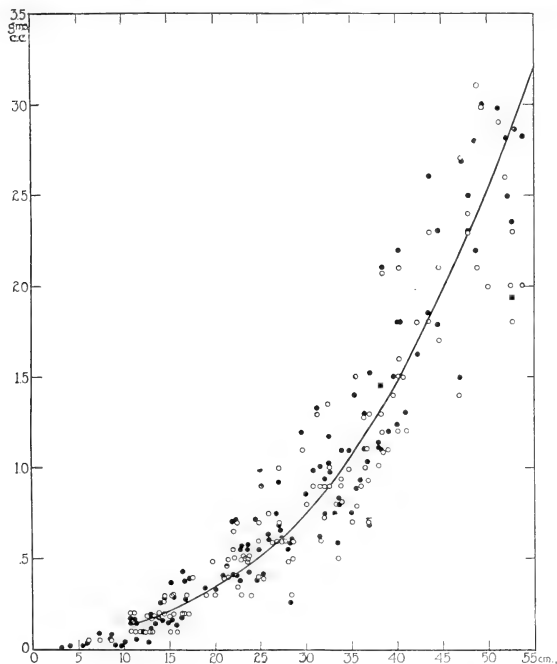


Fig. 21 Field graph and curve of the growth of the spinal cord in weight and volume during fetal life. Abscissa: total body length in cm. Ordinate: spinal cord weight and volume in grams (cc.). Individual cases indicated by solid dots (for weights) and by circles (for volume). Curve drawn to the formula: $Y = 0.01 [(0.17X)^{2.57} + 11.0]$. (Data from tables 33, 34, 35.)

various brain parts form of the encephalon in prenatal life. The consideration of the growth of the fetal brain and the spinal cord in relation to the postnatal changes of these structures is reserved for a later publication. It is hoped also that it will be possible

to present at a later time an outline of the prenatal growth of the various internal parts of the brain for which data are now being collected.

Growth of the central nervous system as a whole

The growth curve of the central nervous system (represented by the volume of the central nervous system, fig. 2) is analogous in both its character and its slope to the growth curves of nearly all the fetal viscera. The abscissa of this curve is the crown-

TABLE 35
Spinal cord volume and weight
(data of Jackson, '09, and of Volpin, '02)

RANGE OF CROWN-HEEL LENGTH	SPINAL-CORD VOLUME (JACKSON, '09)			SPINAL-CORD WEIGHT (VOLPIN, '02)		
	Average crown-heel length	Average cord volume	Number of cases	Average crown-heel length	Average cord weight	Number of cases
<i>cm.</i>	<i>cm.</i>	<i>cc.</i>		<i>cm.</i>	<i>grams</i>	
0 to 5	1.77	0.02225	3			
5 to 10	7.7	0.115	3			
10 to 15	12.3	0.1671	3			
15 to 20	17.1	0.3447	5	16.0	0.75	1
20 to 25	22.4	0.664	4	20.0	1.5	1
25 to 30	27.4	0.908	5	28.0	2.0	1
30 to 35	32.7	1.53	2	32.7	3.0	3
35 to 40	38.5	1.8	1	38.5	3.75	2
40 to 45	44.2	3.3	1	41.8	4.5	3
45 to 50				45.7	6.6	3
50 to 55	51.9	3.04	2	51.0	9.5	3

heel length and represents a simple lineal type of growth. The value of the central nervous system is therefore a function of the crown-heel length which has been raised to approximately the third power. Obviously, this is a typical concave volumetric curve. It is similar in type to all of the visceral volumetric and ponderal curves of growth which have been worked out in this laboratory and of which a summary has been published elsewhere (Scammon, '21). It is also analogous to the curves of fetal body weight and of weights of body parts which have been expressed in functions of the crown-heel length of the fetus. In order to compare

the growth of the central nervous system to the growth of other parts of the body it has been considered as a unit. It is however a complex of four distinct subtypes of growth, all of which are dominated by the growth of the bulky cerebral hemispheres.²

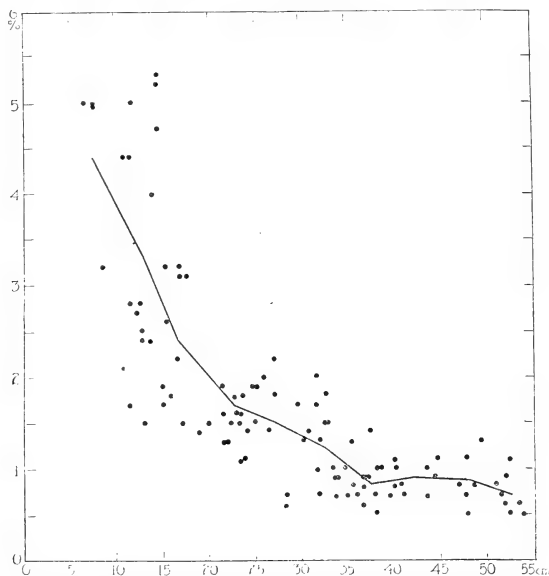


Fig. 22 Field graph and curve (connecting the average points of each 5-cm. crown-heel interval) of the per cent which the spinal cord volume forms of the encephalon volume. Abscissa: total body length in cm. Ordinate: spinal cord in per cent of the encephalon volume. Individual cases indicated by solid dots. (Data from table 40.)

²In order to compare these four types of growth, ten methods were used: 1) the various absolute length curves (fig. 29) were calculated against crown-heel body length (cm.) and reduced to a percentage basis; 2) the absolute volume curves likewise (fig. 30) were taken to crown-heel length and reduced to a per cent basis; 3) the absolute length curves (fig. 33) were calculated for age in fetal months and reduced to a common per cent; 4) the absolute volume curves also (fig. 36) were taken to age in fetal months and reduced to a percentage basis; 5) percentage increments of linear curves (fig. 25) were calculated for 5-cm. C H intervals;

TABLE 36

Length of the spinal cord

Formula: Spinal cord length (cm.) = 10 crown-heel length (cm.)^{.467} - 4
(144 cases)

CROWN-HEEL LENGTH		OBSERVED SPINAL CORD LENGTH			CALCULATED SPINAL CORD LENGTH	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maximum	Minimum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5	3.9	2.5	1.4	1.9	1.4	-0.5	4
5 to 10	7.7	4.0	2.3	3.0	3.6	+0.6	9
10 to 15	12.1	6.2	4.0	4.7	5.4	+0.7	20
15 to 20	16.7	9.7	5.3	7.0	6.9	-0.1	14
20 to 25	22.7	10.7	7.0	8.7	8.6	-0.1	20
25 to 30	26.9	10.7	8.5	9.75	9.6	-0.1	14
30 to 35	32.6	13.3	9.0	10.6	10.9	+0.3	18
35 to 40	37.1	16.5	9.5	12.2	11.8	-0.4	17
40 to 45	42.2	14.0	11.0	12.4	12.8	+0.4	12
45 to 50	48.2	14.8	12.7	13.5	13.9	+0.4	8
50 to 55	52.2	15.8	13.2	14.5	14.6	+0.1	8

TABLE 37

Calculated spinal cord length at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	SPINAL CORD LENGTH	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>per cent</i>	<i>per cent</i>
5	2.2			15.5
10	4.6	2.4	109.0	32.4
15	6.4	1.8	39.2	45.0
20	7.9	1.5	23.4	55.6
25	9.2	1.3	16.4	64.8
30	10.4	1.2	13.0	73.3
35	11.4	1.0	9.6	80.3
40	12.4	1.0	8.2	87.3
45	13.3	0.9	7.5	94.0
50	14.2	0.9	6.5	100.0
55	15.0	0.8	5.9	105.6

6) percentage increments of volume curves (fig. 26) were determined for 5-cm. intervals; 7) percentage increments of linear curves (fig. 27) were calculated for age in fetal months; 8) percentage increments of linear curves (fig. 28) were determined for age in fetal months; 9) the various linear formulae of the respective absolute curves (table 43), and, 10) the volumetric formulae of the absolute volume curves (table 43) were compared.

The first type, cerebral growth, is characterized by a constant growth in linear measurements from the second fetal month until birth, and shows a steady and relatively slow increase in volume

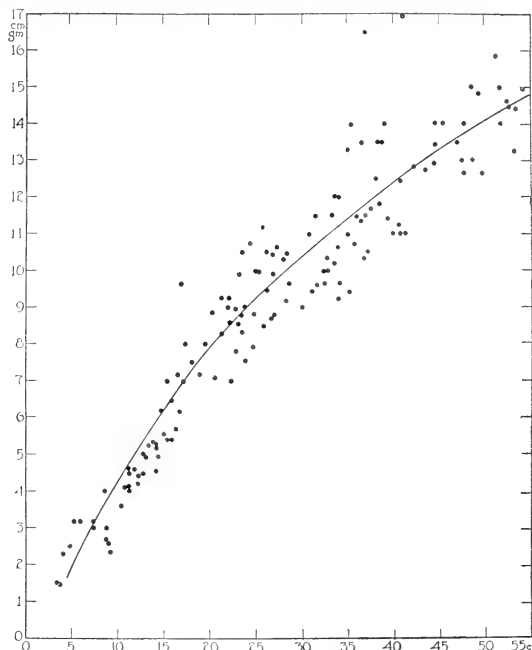


Fig. 23 Field graph and curve of the growth of the spinal cord in fetal life as shown by the spinal cord length. Abscissa: total body length in cm. Ordinate: spinal cord length in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = (10.0X)^{.467} - 4.0$. (Data from tables 36 and 37.)

before the sixth fetal month and a constant but more rapid increase from that time until birth. Brain stem and cord growth, on the other hand, advances much faster previous to the seventh fetal month than it does in the last four months of fetal life. Cerebellum growth, on the contrary, proceeds slowly until the

seventh fetal month and from that time until birth it exceeds all other growth activity in the central nervous system. The last type, compound growth, represents merely the combined effect of two or more of the above types predominated by the mass of the cerebral hemispheres. These varieties of growth will now be considered in more detail.

TABLE 38

Combined brain-cord length

Formula: Brain-cord length (cm.) = (10 crown-heel length (cm.))^{.514} - 4
(137 cases)

CROWN-HEEL LENGTH		OBSERVED BRAIN-CORD LENGTH			CALCULATED BRAIN-CORD LENGTH	DIFFERENCE BETWEEN OBSERVED AND CALCULATED MEANS	NUMBER OF CASES
Range	Mean	Maxi- mum	Mini- mum	Mean			
cm.	cm.	cm.	cm.	cm.	cm.	cm.	
0 to 5	4.0	4.9	3.1	3.0	2.7	-0.3	3
5 to 10	7.1	5.7	4.0	4.4	4.9	+0.5	7
10 to 15	12.1	8.5	5.0	6.9	7.8	+0.9	20
15 to 20	16.6	12.7	7.9	9.8	9.8	0	14
20 to 25	22.6	14.7	10.2	12.4	12.2	-0.2	19
25 to 30	26.9	15.1	12.1	13.8	13.8	0	13
30 to 35	32.8	18.4	14.1	15.4	15.6	-0.2	16
35 to 40	37.1	20.2	13.5	17.4	16.9	-0.5	17
40 to 45	42.2	19.7	16.1	18.1	18.4	-0.3	11
45 to 50	48.2	21.7	18.8	19.8	19.9	+0.1	9
50 to 55	52.2	23.1	20.4	21.3	20.9	-0.4	8

TABLE 39

Calculated combined brain-cord length at 5-cm. intervals of crown-heel length

CROWN-HEEL LENGTH	BRAIN-CORD LENGTH	INCREMENT IN EACH 5-CM. INTERVAL		RATIO TO NEW-BORN
		cm.	per cent	
cm.	cm.			per cent
5	3.5			17.2
10	6.6	3.1	88.5	32.3
15	9.2	2.6	39.4	45.1
20	11.2	2.0	21.7	54.9
25	13.1	1.9	17.0	64.2
30	14.8	1.7	13.0	72.5
35	16.3	1.5	10.1	79.9
40	17.8	1.5	9.2	87.2
45	19.1	1.3	7.3	93.6
50	20.4	1.3	6.4	100.0
55	21.6	1.2	5.9	106.0

Cerebral growth. The growth of the cerebrum is characterized by a steady absolute increment in all of its linear measurements from the second fetal month until birth. This can be observed by the inspection of the curves of the fronto-occipital length and the temporal diameter (fig. 29, curves I and II, respectively).

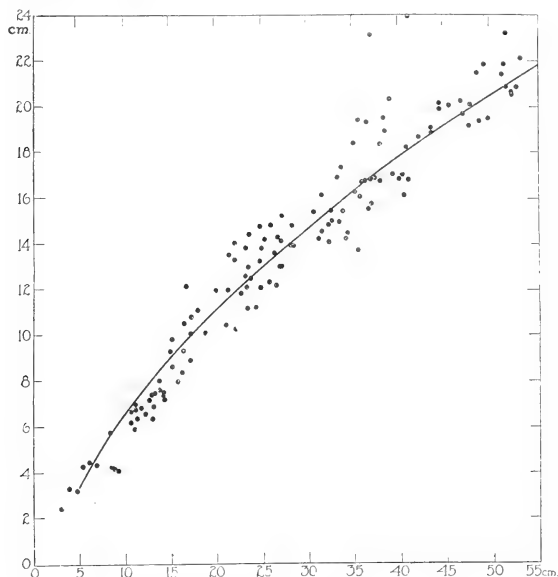


Fig. 24 Field graph and curve of the growth of the spinal cord and brain stem in fetal life as shown by the brain-cord length. Abscissa: total body length in cm. Ordinate: brain-cord length in cm. Individual cases indicated by solid dots. Curve drawn to the formula: $Y = (10.0X)^{.514} - 4.0$. (Data from tables 38 and 39.)

These curves are both straight lines and lie intermediate to the vermis cerebelli absolute curves below and to the curve of the spinal cord above. When calculated against time in fetal months (fig. 33, curves I and II), the fronto-occipital length and the

(Text continued on page 479)

TABLE 40

Ratios of the volumes of the various parts of the central nervous system to the entire brain volume

CROWN-HEEL LENGTH RANGE	RATIO OF:				
	Spinal cord	Pons and medulla	Midbrain	Cerebellum	Cerebral hemispheres
<i>cm.</i>					
5 to 10	4.70				
10 to 15	3.30	5.5	2.90	3.0	87.7
15 to 20	2.40	3.6	2.40	3.0	90.9
20 to 25	1.70	2.7	1.60	3.1	93.1
25 to 30	1.50	2.2	1.40	3.0	94.0
30 to 35	1.20	1.9	0.96	2.8	94.2
35 to 40	0.81	1.7	0.90	3.8	94.1
40 to 45	0.90	1.6	0.87	4.4	93.2
45 to 50	0.84	1.5	0.70	6.2	92.0
50 to 55	0.70	1.4	0.67	5.8	91.8

TABLE 41

COMPARISON OF THE WEIGHTS AND VOLUMES
OF THE SPINAL CORD, THE ENTIRE BRAIN AND THE VARIOUS PARTS OF THE BRAIN
IN THE FETAL PERIOD

	AVERAGE WEIGHT (GM) AND VOLUME (CC) IN EACH 5 CM. INTERVAL (CH.)													
		0 to 5	5 to 10	10 to 15	15 to 20	20 to 25	25 to 30	30 to 35	35 to 40	40 to 45	45 to 50	50 to 55		
Spinal Cord (128 cases)	Wt.	0.05	0.05	0.15	0.29	0.51	0.69	0.92	1.23	1.6	2.4	2.9		
	Vol.	—	0.05	0.17	0.28	0.48	0.65	0.88	1.14	1.7	2.4	2.5		
Pons and Medulla (109 cases)	Wt.	—	—	0.35	0.47	0.82	0.92	1.7	2.6	3.2	4.4	5.4		
	Vol.	—	—	0.34	0.44	0.77	0.92	1.5	2.4	3.1	4.5	5.2		
Mid brain (109 cases)	Wt.	—	—	0.22	0.32	0.50	0.64	0.87	1.2	1.6	2.4	2.6		
	Vol.	—	—	0.19	0.30	0.47	0.62	0.81	1.4	1.6	2.4	2.4		
Cerebellum (115 cases)	Wt.	—	—	0.25	0.43	1.02	1.39	2.50	5.6	9.2	18.9	21.9		
	Vol.	—	—	0.26	0.41	0.95	1.34	2.36	5.4	8.9	18.9	21.3		
Right Cerebral Hemisphere (115 cases)	Wt.	—	—	2.8	6.2	15.1	22.4	39.2	66.0	94.0	142.0	174.0		
	Vol.	—	—	2.6	6.0	14.6	21.7	38.2	64.1	92.2	136.0	170.0		
Left Cerebral Hemisphere (114 cases)	Wt.	—	—	2.9	6.2	14.6	22.5	40.0	66.0	97.5	145.2	171.8		
	Vol.	—	—	2.6	6.0	14.2	21.6	39.2	65.8	94.6	139.0	166.0		
Entire Brain (144 cases)	Wt.	0.3	1.3	5.6	12.6	29.9	47.8	82.9	143.6	196.2	313.6	376.1		
	Vol.	0.3	1.2	5.4	11.9	30.8	49.8	76.5	132.0	191.2	299.1	364.9		
Central Nervous System (146 cases)	Wt.	0.33	1.4	5.8	12.9	32.4	48.5	83.8	144.8	198.0	316.0	379.0		
	Vol.	0.32	1.3	5.4	12.5	31.1	46.9	81.8	141.8	197.7	306.0	367.5		

TABLE 42

CALCULATED ABSOLUTE AND PERCENTAGE INCREMENTS OF THE SEVERAL PARTS OF THE BRAIN AND OF THE SPINAL CORD IN EACH MONTH OF THE FETAL PERIOD

Age According to MALL'S TABLES (FETAL Months)	CEREBRUM						CEREBELLUM						PONS AND MEDULLA						MIDBRAIN		SPINAL CORD			
	Both Hemispheres Volume		Fronto- occipital Length		Temporal Diameter		Cerebellum Volume		Vermis Cerebelli Length		Vermis Cerebelli Height		Pons and Medulla Volume		Pons Length		Midbrain Volume		Spinal Cord Volume		Spinal Cord Length			
	cc	%	cm.	%	cm.	%	c.c.	%	cm.	%	cm.	%	cc	%	cm.	%	c.c.	%	cc	%	cm.	%		
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
2	ca. 0.04	—	1.077	—	0.71	—	0.20	—	0.22	—	0.05	—	0.20	—	0.24	—	0.12	—	0.04	—	0.90	—		
3	168 450	ca. 1.96	153.0	1.65	131	0.27	35	0.40	81.8	0.23	36.0	0.26	300	0.42	74.8	0.16	33.3	0.15	36.4	4.51	401			
4	1167	390	340	730	2.70	68.5	0.34	30	0.74	85.0	0.52	126	0.51	962	0.63	51.6	0.29	81.3	0.29	93.3	7.30	62		
5	3327	105	4.62	3.60	3.75	34.7	0.89	161.5	1.09	47.3	0.82	57.6	0.93	82.4	0.82	29.1	0.51	75.8	0.52	79.3	9.18	25.0		
6	6933	109	5.76	24.6	4.65	23.9	2.25	153.0	1.45	33.0	1.13	37.8	1.56	67.8	0.99	20.9	0.83	62.8	0.86	65.4	10.68	16.3		
7	1172	68.5	6.74	17.0	5.42	16.7	4.99	121.9	1.78	22.8	1.41	24.7	2.31	48.0	1.14	14.9	1.20	44.6	1.25	45.4	11.84	10.9		
8	1808	54.2	7.69	14.0	6.16	13.8	9.53	91.0	2.13	13.7	1.70	20.6	3.23	39.8	1.28	12.5	1.05	37.5	1.72	37.6	12.88	8.8		
9	2892	37.8	8.47	10.2	6.79	10.1	15.84	66.3	2.43	14.1	1.95	14.7	4.17	29.1	1.34	9.3	2.10	27.3	2.20	27.9	13.70	6.4		
10	2016	35	9.00	9.5	7.20	9.2	20.89	54.2	2.64	12.4	2.13	13.2	48.8	27.5	14.7	7.3	24.4	2.5	2.56	26	14.21	5.7		

TABLE 43

EMPIRICAL FORMULAE REPRESENTING CURVES OF LINEAR AND VOLUMETRIC GROWTH OF THE CENTRAL NERVOUS SYSTEM IN THE FETAL PERIOD

MEASUREMENT	SPECIFIC FORMULAE (EXPONENTIAL)	TYPE OF FORMULA	SPECIFIC FORMULAE (LOGARITHMIC)	TYPE OF FORMULA
Fronto-occipital Length	$Y = 0.175X + 0.25$	$Y = aX + b$	$Y = 0.175X + 0.25$	$Y = aX + b$
Temporal-temporal Diameter	$Y = 0.138X + 0.3$		$Y = 0.138X + 0.3$	
Pons Length	$Y = 0.0263X + 0.16$		$Y = 0.0263X + 0.16$	
Calliculi Length	$Y = 0.015X + 0.47$		$Y = 0.015X + 0.47$	
Spinal Cord Length	$Y = (10.0X)^{0.467} - 4.0$	$Y = (aX)^b - c$	$Y = 0.07X + 10/\log X - 6.5$	$Y = aX + b/\log X - c$
Brain Cord Length	$Y = (10.0X)^{0.514} - 4.0$		$Y = 0.17X + 11/\log X - 6.7$	
Fronto-spinal Length	$Y = (2.0X)^{0.47} - 2.2$		$Y = 0.051X + 4/\log X - 2.85$	
Vermis Cerebelli Length	$Y = 0.01(X^{1.41} + 15.0)$	$Y = 0.01(X^a + b)$	$Y = 0.01(0.073X + 2.85)^3$	$Y = 0.01(aX + b)^3$
Vermis Cerebelli Height	$Y = 0.01(X^{1.37})$		$Y = 0.01(0.07X + 2.45)^3$	
Spinal Cord Volume	$Y = 0.01[(0.17X)^{2.57} + 11.0]$	$Y = 0.01[(aX)^b + c]$	$Y = 1.4(0.02X + 0.31)^3 - 0.013X + 0.1$	$Y = a(bX + c)^3 \pm dX + e$
Pons and Medulla Volume	$Y = 0.01(0.2X)^{2.67} + 20.0$		$Y = 0.94(0.03X + 0.2)^3 + 0.1$	
Mid Brain Volume	$Y = 0.01[(0.16X)^{2.56} + 12.0]$		$Y = 1.5(0.02X + 0.31)^3 - 0.027X + 0.36$	
Cerebellum Volume	$Y = 0.01[(0.095X)^{4.9} + 20.0]$		$Y = 0.01(0.025X + 1.03)^3$	
Right Hemisphere Volume	$Y = (0.1X)^{2.15}$	$Y = (aX)^b$	$Y = (0.1085X - 0.09)^3$	$Y = a(bX - c)^3$
Left Hemisphere Volume	$Y = (0.105X)^{2.04}$		$Y = (0.108X - 0.05)^3$	
Both Hemispheres Volume	$Y = (0.12X)^{2.19}$			
Encephalon Volume	$Y = (0.125X)^{2.16} + 15$	$Y = (aX)^b + c$	$Y = 1.1(0.137X - 0.07)^3 - 0.4X + 5.0$	$Y = a(bX \pm c)^3 - dX + e$
Encephalon Weight	$Y = (0.13X)^{2.10} + 1.0$		$Y = 1.15(0.14X + 0.2)^3 - X + 10.0$	
Central Nervous System Volume	$Y = (0.114X)^{2.34} + 2.0$		$Y = 1.17(0.137X - 0.03)^3 - 1.12X + 16.0$	

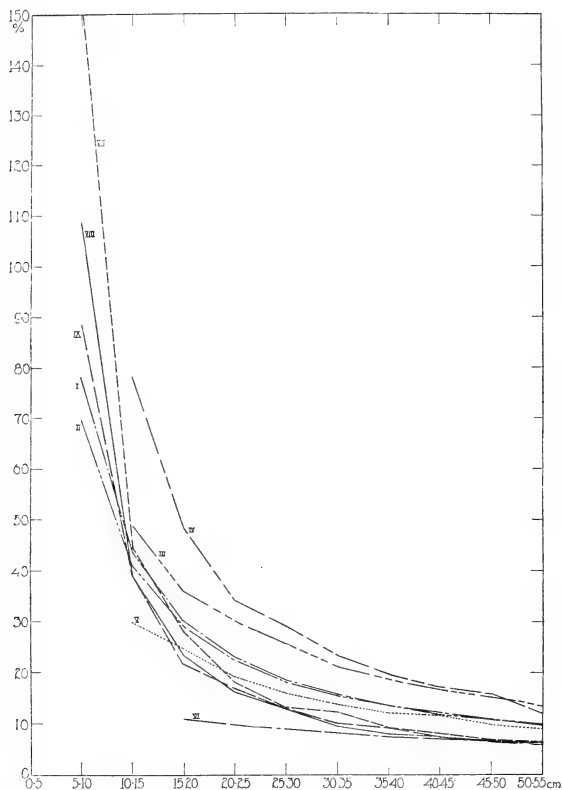


Fig. 25 A series of curves illustrating the percentage increments of the various linear dimensions of the brain, brain parts, and spinal cord. Abscissa: total body length in cm. Ordinates: percentage increments calculated from the formulae of the respective absolute curves. (Data from tables 14, 16, 20, 22, 26, 30, 32, 37, and 39.) I, percentage increment of fronto-occipital diameter. II, percentage increment of temporal diameter. III, percentage increment of vermis cerebelli length. IV, percentage increment of vermis cerebelli height. V, percentage increment of pons length. VI, percentage increment of colliculi length. VII, percentage increment of frontospinal length. VIII, percentage increment of spinal cord length. IX, percentage increment of brain-cord length.

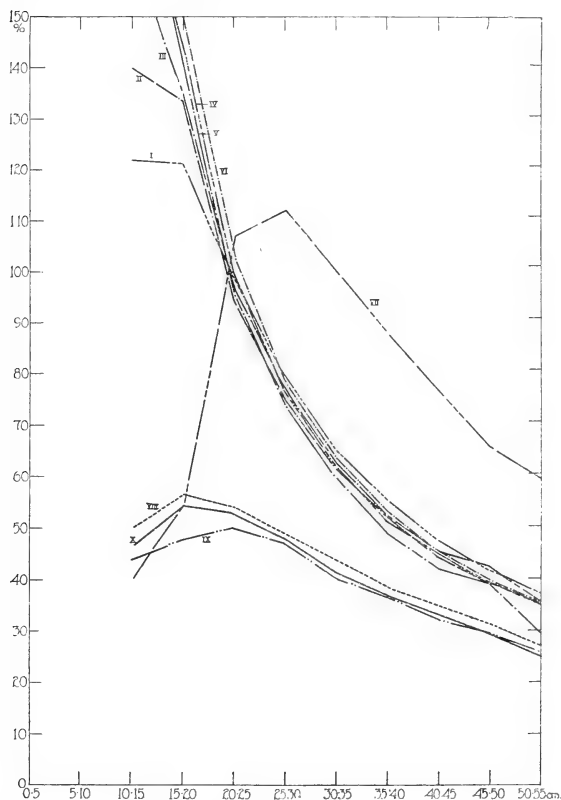


Fig. 26 A series of curves illustrating the percentage increments of the various volumetric determinations of the brain, brain parts, and spinal cord. Abscissa: total body length in cm. Ordinates: percentage increments calculated from the formulae of the respective absolute growth curves. (Data from tables 2, 4, 5, 8, 10, 12, 18, 24, 28, and 34.) I, percentage increment of central nervous system volume. II, percentage increment of encephalon volume. III, percentage increment of entire brain weight by literature. IV, percentage increment of right hemisphere volume. V, percentage increment of left hemisphere volume. VI, percentage increment of both hemisphere volume. VII, percentage increment of cerebellum volume. VIII, percentage increment of pons and medulla volume. IX, percentage increment of midbrain volume. X, percentage increment of spinal cord volume.

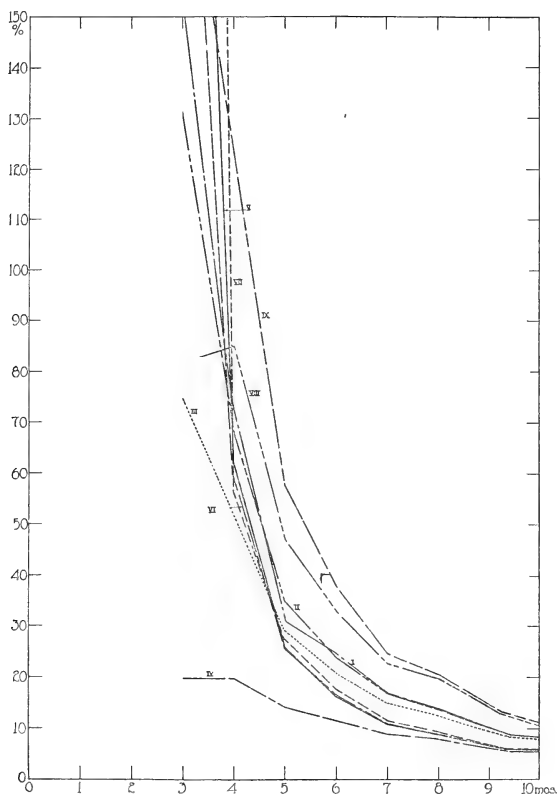


Fig. 27 A series of curves illustrating the percentage increments of the various linear dimensions of the brain, brain parts, and spinal cord when interpreted in time in fetal months (as determined by Mall's convention). Abscissa: total time of fetal life in months. Ordinate: percentage increments calculated from the formulae of corresponding absolute curves and transposed into time. (Data from table 41.) I, percentage increment of fronto-occipital diameter. II, percentage increment of temporal diameter. III, percentage increment of pons length. IV, percentage increment of colliculi length. V, percentage increment of spinal cord length. VI, percentage increment of brain-cord length. VII, percentage increment of frontospinal length. VIII, percentage increment of vermis cerebelli length. IX, percentage increment of vermis cerebelli height.

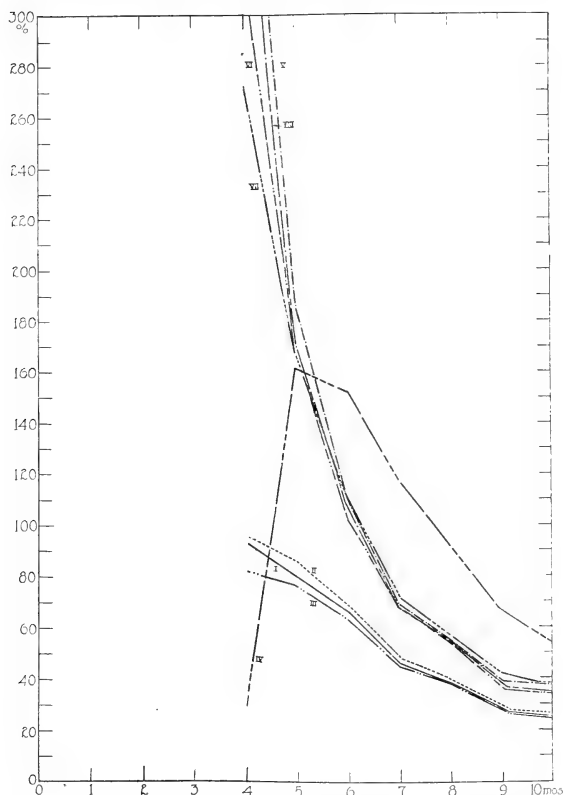


Fig. 28 A series of curves illustrating the percentage increment of the various volumetric determinations of the brain, brain parts, and spinal cord. Abscissa: total time in fetal months as determined by Mall's convention. Ordinates: percentage increments calculated from the formulae of respective absolute growth curves. (Data from tables 2, 4, 5, 8, 10, 12, 18, 24, 28, and 34.) I, percentage increment of spinal cord volume. II, percentage increment of pons and medulla volume. III, percentage increment of midbrain volume. IV, percentage increment of cerebellum volume. V, percentage increment of both hemisphere volume. VI, percentage increment of the encephalon volume. VII, percentage increment of the volume of the central nervous system. VIII, percentage increment of the encephalon weight (cases collected from the literature).

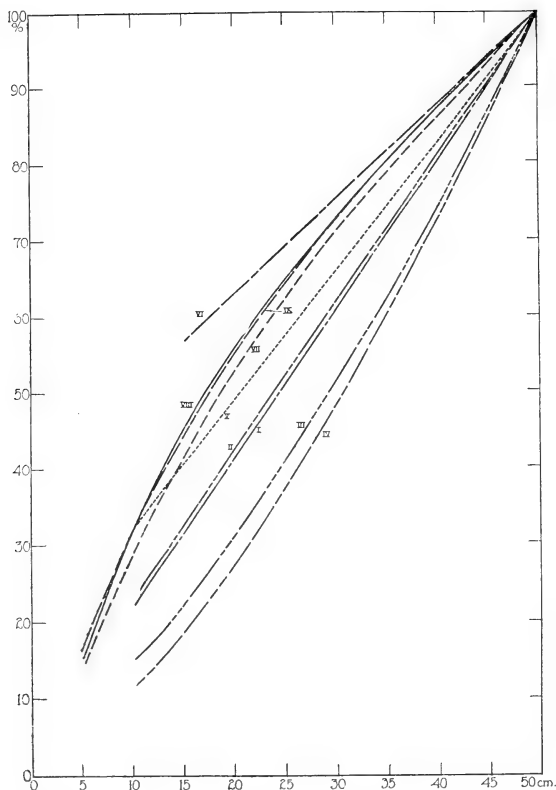


Fig. 29 A series of curves illustrating the growth (in linear dimensions) of the brain, brain parts, and spinal cord in per cent of the respective new-born linear measurements. Abscissa: total body length in cm. Ordinates: linear dimensions calculated in per cents of the respective new-born measurements. Data from the formulae in table 43. I, frontal-occipital diameter. II, temporal diameter. III, vermis cerebelli length. IV, vermis cerebelli height. V, pons length. VI, colliculi length. VII, frontospinal length. VIII, spinal cord length. IX, brain-cord length.

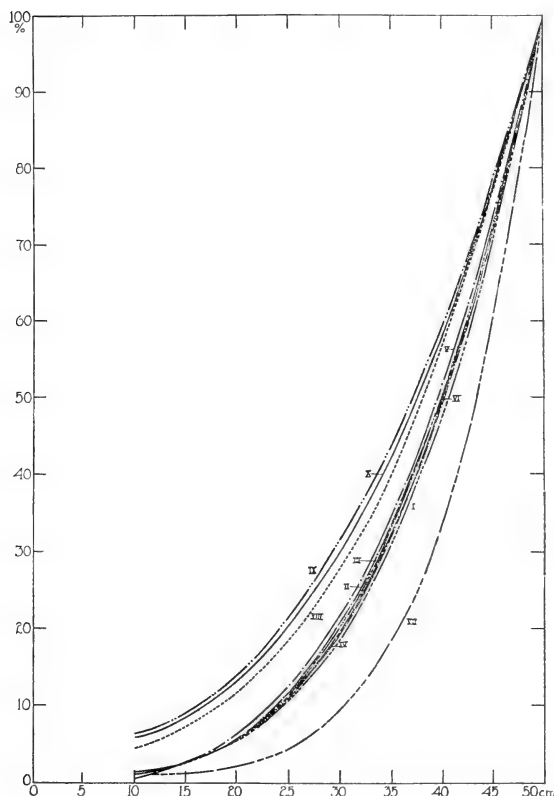


Fig. 30 A series of curves illustrating the growth in the volumes of the brain, brain parts, and spinal cord in per cent of the respective new-born volumetric determinations. Abscissa: total body length in cm. Ordinate: volumes calculated in per cents of the respective new-born volumetric determinations. (Data from the formulae in table 43.) I, volume of the central nervous system. II, encephalon volume. III, encephalon weight by cases from the literature. IV, right hemisphere volume. V, left hemisphere volume. VI, both hemisphere volumes. VII, cerebellum volume. VIII, pons and medulla volume. IX, midbrain volume. X, spinal-cord volume.

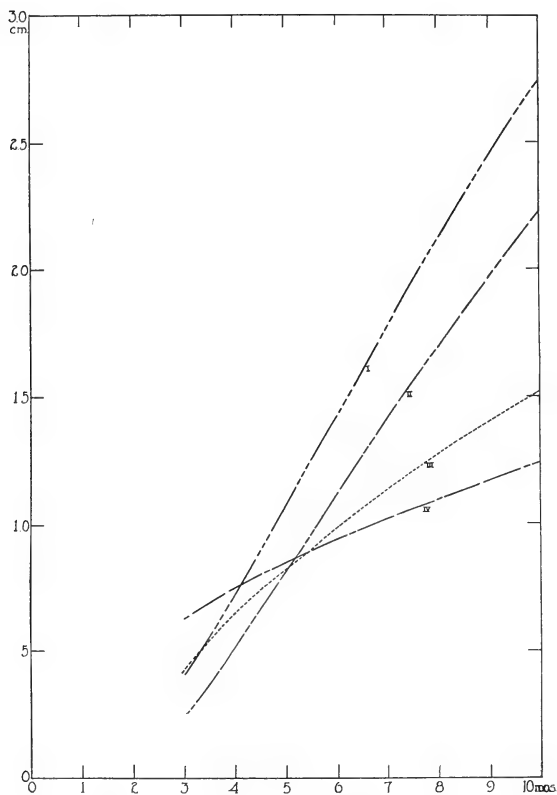


Fig. 31 A series of curves illustrating the growth of the pons, colliculi, and vermis cerebelli by linear measurements. Abscissa: fetal months (crown-heel reduced to time by Mall's convention). Ordinates: lengths of pons, colliculi, and vermis cerebelli in cm. (Data from formulae in table 43.) I, vermis cerebelli length. II, vermis cerebelli height. III, pons length. IV, colliculi length.

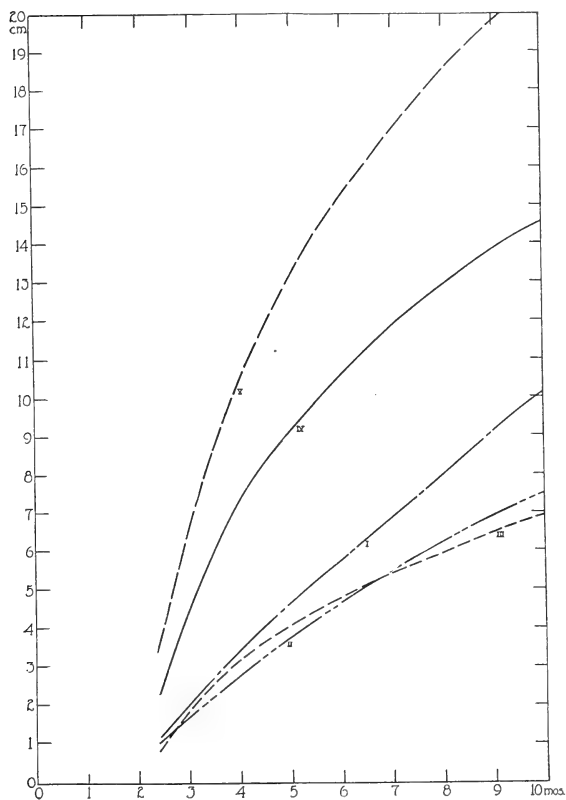


Fig. 32 A series of curves illustrating by linear measurements the growth of the cerebrum, brain stem, and spinal cord. Abscissa: fetal months (crown-heel reduced to time by Mall's convention). Ordinates: lengths of the cerebrum, brain stem, and spinal cord in cm. (Data from formulae in table 43.) I, fronto-occipital diameter. II, temporal diameter. III, frontospinal length. IV, spinal cord length. V, brain-cord length.

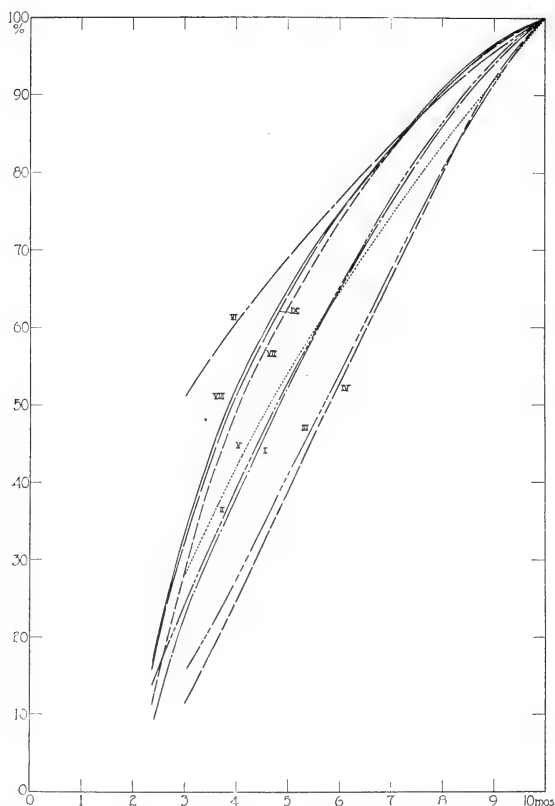


Fig. 33 A series of curves illustrating the growth (in linear dimensions) of the brain, brain parts, and spinal cord calculated in per cents of the respective new-born linear measurements. Abscissa: fetal months (crown-heel length reduced to time by Mall's convention). Ordinates: linear dimensions calculated in per cents of the respective new-born measurements. (Data from the formulae in table 43.) I, fronto-occipital diameter. II, temporal diameter. III, vermis cerebelli length. IV, vermis cerebelli height. V, pon length. VI, colliculi length. VII, frontospinal length. VIII, spinal-cord length. IX, brain-cord length.

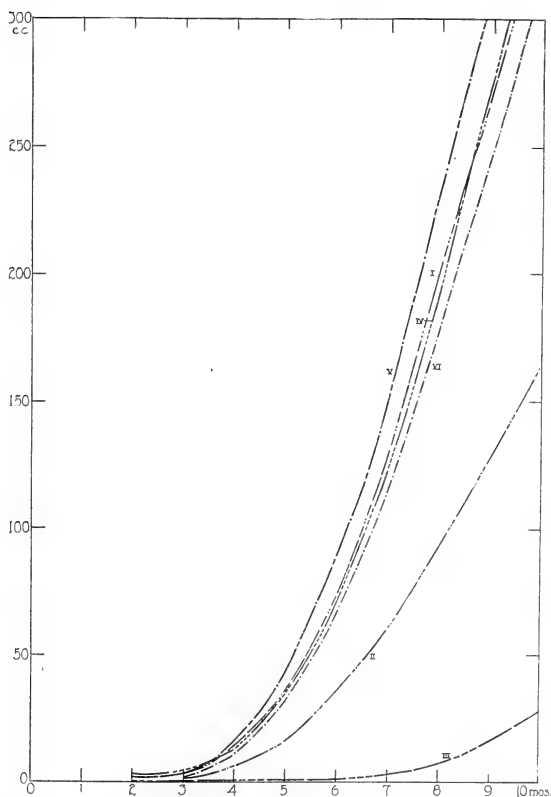


Fig. 34 A series of curves illustrating the growth of the cerebrum and cerebellum as shown by volumetric determinations. Abscissa: fetal months (crown-heel length reduced to time by Mall's convention). Ordinates: volumes of the encephalon, right hemisphere, and cerebellum. (Data from formulae in table 43.) I, encephalon volume. II, right hemisphere volume. III, cerebellum volume. IV, central nervous system volume. V, encephalon weight (data collected from the literature). VI, both hemisphere volume.

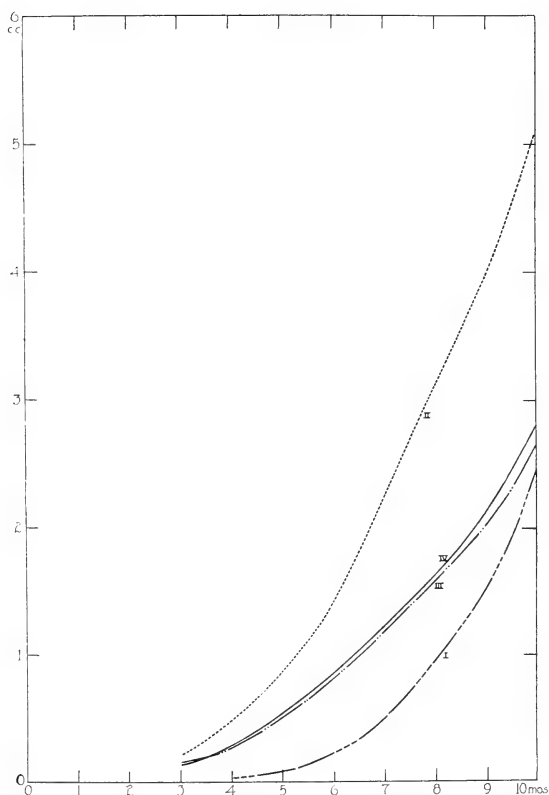


Fig. 35 A series of curves illustrating the growth of the cerebellum, pons and medulla, midbrain, and spinal cord. Abscissa: fetal months (crown-heel reduced to time by Mall's convention). Ordinates: volumes of the cerebellum, pons and medulla, midbrain, and spinal cord. (Data from the formulae in table 43.) I, cerebellum volume. II, pons and medulla volume, III, midbrain volume. IV, spinal cord volume.

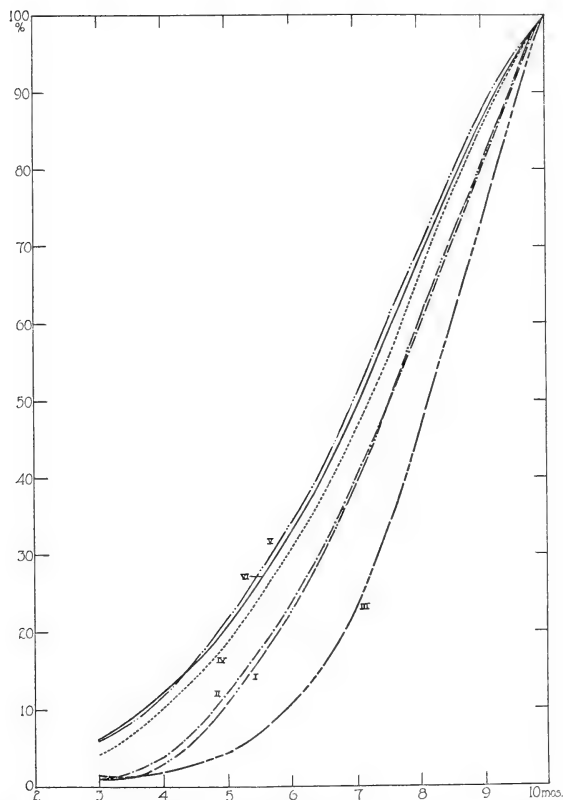


Fig. 36 A series of curves illustrating growth of the brain, brain parts, and spinal cord in per cent of the respective new-born volumetric determinations. Abscissa: fetal months (crown-heel reduced to time by Mall's convention). Ordinates: per cents of volumes as compared with the respective new-born volumetric determinations. (Data from the formulae in table 43.) I, encephalon volume. II, cerebral hemispheres volume. III, cerebellum volume. IV, pons and medulla volume. V, midbrain volume. VI, spinal cord volume.

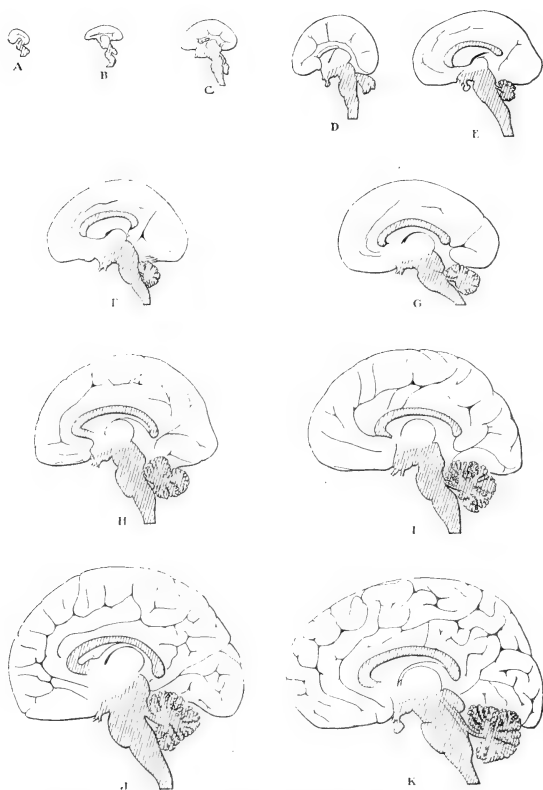


Fig. 37 A series of midsagittal tracings of the brain and brain stem. Each figure represents an average brain in each 5-cm. crown-heel interval. A, ca. 4 cm. C H length. B, ca. 7.5 cm. C H length. C, ca. 2.5 cm. C H length. D, ca. 17.5 cm. C H length. E, ca. 22.5 cm. C H length. F, ca. 27.5 cm. C H length. G, ca. 32.5 cm. C H length. H, ca. 37.5 cm. C H length. I, ca. 42.5 cm. C H length. J, ca. 47.5 cm. C H length. K, ca. 52.5 cm. C H length.

temporal diameter are practically identical. They occupy the same characteristic intermediate position between the cerebellum and the brain stem and spinal cord absolute growth curves. The rate of growth of the fronto-occipital diameter, when calculated from 5-cm. C H intervals (fig. 25, curves I and II) and also when calculated for age in fetal months (fig. 27, curves I and II), portrays an intermediate percentage increment curve lying between the cerebellum above and the brain stem below. The formulae of the fronto-occipital length and the temporal diameter (table 43) likewise show a characteristic grouping of the hemispheres to the general formula: $Y = aX + b$, in which Y is the fronto-occipital length or the temporal diameter in cm., X is the crown-heel length in cm., and a and b are constants determined for the two formulae.

Cerebral growth in volume shows a steady and relatively slow increase before the sixth fetal month and then a constant but more rapid increase from that time until birth. This is indicated by the right hemisphere volume, left hemisphere volume, and both hemisphere volume (figs. 4, 5, and 6, respectively). When calculated against crown-heel length and reduced to percentage basis (fig. 30), the right hemisphere volume, left hemisphere volume, and the volume of both hemispheres (curves IV, V, and VI, respectively), as well as the absolute curves of the central nervous system and the total brain volume (curves I and II, respectively), fall practically upon one another. The cerebellum volume curve lies below and the brain stem and spinal cord volume curve above the curves dominated by the factor of cerebral growth. Upon inspection of the curve of both hemisphere volume when calculated against time in fetal months and reduced to a percentage basis (fig. 36, curve II), a similar central grouping between the cerebellum volume, the brain stem volume, and spinal cord volume is observed. Turning to the figures upon the rate of growth of the cerebrum, which is determined for 5-cm. intervals (fig. 26, curves IV, V, and VI) and for age in fetal months (fig. 28, curve V), one observes the unswerving tendency of the cerebral growth to proceed faster before the sixth month and slower thereafter.

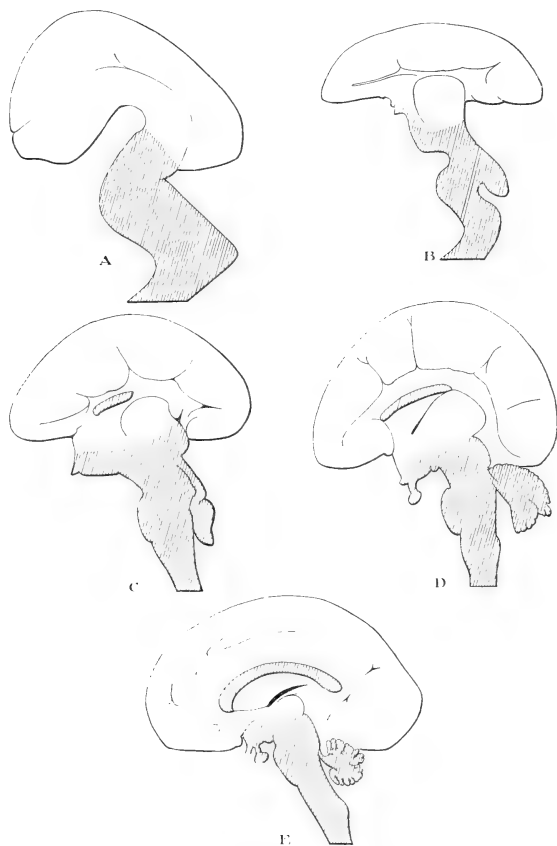
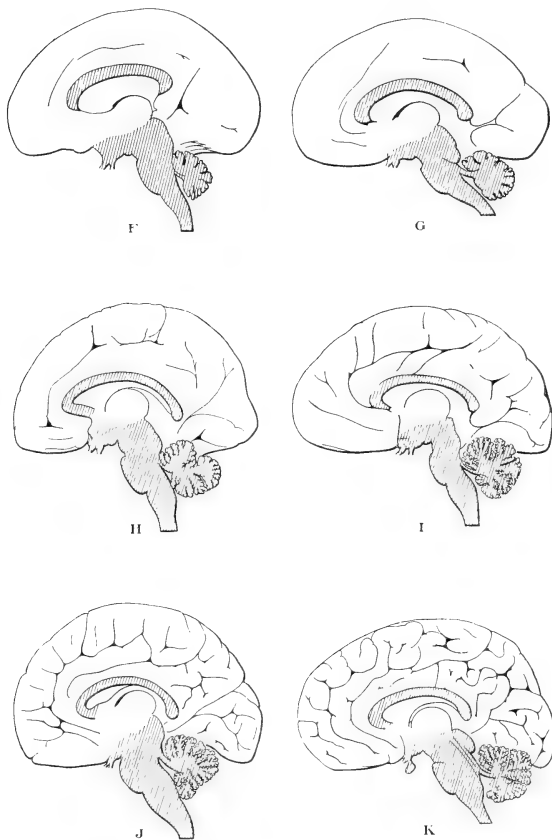


Fig. 38 A series of midsagittal tracings (of the specimens shown in figure 35) drawn to a standard fronto-occipital length. A, ca. 4 cm. C H length. B, ca. 7.5 cm. C H length. C, ca. 12.5 cm. C H length. D, ca. 17.5 cm. C H length.



E, ca. 22.5 cm. C H length. F, ca. 27.5 cm. C H length. G, ca. 32.5 cm. C H length. H, ca. 37.5 cm. C H length. I, ca. 42.5 cm. C H length. J, ca. 47.5 cm. C H length. K, ca. 52.5 cm. C H length.

The volumetric formulae of the right hemisphere volume, left hemisphere volume, and both hemisphere volume (table 43) are all expressed by the simple formula:

$$Y = (aX)^b$$

in which Y is the value to be determined in cc.; X , the crown-heel length in cm., and a and b are constants for the respective volumes.

Brain stem and spinal cord growth. The growth of the brain stem and spinal cord, as shown by curves of absolute linear dimensions and absolute volumes, represents a second definite type of growth in the central nervous system. It is characterized by a relatively rapid absolute and relative increase previous to the sixth fetal month and a slow increase during the last four months of intra-uterine life.

The linear determinations showing this type of growth are the frontospinal length, the spinal cord length, and the brain-cord length. When plotted against crown-heel length in centimeters and reduced to percentages of new-born values (fig. 29, curves VII, VIII, and IX) these three curves approximate one another and ascend more rapidly prior to the sixth fetal month than they do in the last four months. They are distinctly separated from the cerebral type of growth and, when calculated in fetal months and reduced to a per cent of the new-born (fig. 33, curves VII, VIII, and IX, respectively), they show even more clearly the almost identical path which they all follow. When calculated to time, they likewise show a rapid increase to the fifth fetal month and a comparatively slow one thereafter. The rates of growth of the brain stem and the spinal cord as shown by these three linear determinations can be observed equally well when taken for 5 cm. intervals (fig. 25, curves VII, VIII, and IX, respectively), or when calculated for age in fetal months (fig. 27, curves VII, V, and VI, respectively). The brain stem and spinal cord grow at a slower rate both relatively and absolutely than do all other brain parts during the last four months of fetal life.

The classification indicated by comparison of the absolute linear curves is substantiated by a glance at their respective

formulae (table 43). The growths of most of the group are expressed by the general empirical formula:

$$Y = (aX)^b - c$$

in which Y is the brain-part value in cm., X is crown-heel length in cm., and a , b , and c are constants determined separately in each case for the frontal spinal length, spinal cord length, and the brain-cord length.

Two other straight-line measurements, one of the colliculi length and the other of the pons length, do not follow the typical brain stem and spinal cord type of growth for some unknown reason. They are straight-line curves and evidently express the growth of some specific factor rather than the growth of the brain stem of which they are a part. .

The brain stem and cord growth are portrayed also in the curves of the spinal cord volume, the pons and medulla volume, and the midbrain volume. When calculated against crown-heel length (fig. 30, curves X, VIII, and XI, respectively), these volume curves show a relatively rapid absolute growth previous to the sixth month and a slow growth in the last four months of fetal life. They also approximate each other and fall definitely above the cerebral type of growth curve. They are practically identical when calculated against fetal months and reduced to a per cent of the new-born value (fig. 36, curves VI, IV and V, respectively). When the percentage increments of the spinal cord volume, the pons and medulla volume, and the midbrain volume are compared (either when calculated for 5-cm. C H intervals (fig. 26, curves X, VIII, and IX, respectively) or for age in fetal months (fig. 28, curves I, II, and III, respectively)), the distinctive types of the brain stem and spinal cord growth are observed. They show a slow rate of relative growth in the third month, which increases three-fold in rapidity in the fourth month. From this time until birth the growth rate decreases in rapidity so that these portions show a less rapid relative increase in the last three fetal months than does any other brain part.

The classification is indicated also by volumetric formulae (table 43) which can be expressed by the general equation:

$$Y = 0.01 [(aX)^b + c]$$

in which Y is the value desired in cc., X is the crown-heel in cm., and a , b , and c , are constants determined for the spinal-cord volume, the pons and medulla volume, and the midbrain volume.

Cerebellum growth. Cerebellum growth, as shown by linear and volume absolute curves, proceeds slowly until the sixth fetal month, and from that time until birth outstrips all other growth activities in the central nervous system.

The lineal determinations expressing this type of growth are the vermis cerebelli length and the vermis cerebelli height (fig. 29, curves III and IV). If plotted against crown-heel length and reduced to per cents of their new-born values, they lie close together and ascend slowly at first and then more rapidly to birth in very shallow concave curves. They are decidedly different from all other straight-line absolute curves of the nervous system, resembling volumetric curves rather than lineal progressions. They show a similar definite classification when calculated against fetal months (fig. 33, curves III and IV). The rate of growth of the cerebellum, as shown by the percentage increment curves of the vermis cerebelli length and the vermis cerebelli height either when calculated for 5-cm. C H intervals (fig. 25, curves III and IV) or for age in fetal months (fig. 27, curves VIII and IX), reflect the cerebellum type of growth. During the last four fetal months the percentage increment of the vermis cerebelli length and the vermis cerebelli height is higher by 8 to 10 per cent than all other linear percentage increment curves of the central nervous system.

A consideration of the vermis cerebelli length and the vermis cerebelli height formulae (table 43) shows the volumetric tendency of these linear formulae. They can be expressed by the general empirical formula:

$$Y = 0.01 (X^a + b)$$

in which Y is the value desired in cm., X is crown-heel in cm., and a and b are constants determined for both the vermis cerebelli length and the vermis cerebelli height.

The curve of the volume of the cerebellum is striking in character and demonstrates the slow growth of the structure prior

to the sixth fetal month and the tremendously rapid increment from that time until birth. When calculated against crown-heel length (fig. 30, curve VII), and also when calculated against time in fetal months (fig. 36, curve III), the distinct character of the cerebellum volume is apparent. The formula of the growth curve of the cerebellum (table 43) is also characteristic, although it falls with the same general formula as the brain stem and spinal-cord volume, viz.:

$$Y = 0.01 [(aX)^b + c]$$

However, it will be noticed that the b factor is 4.9 in the case of the cerebellum volume. This means that while the spinal cord volume, midbrain volume, and the pons and medulla volume are increasing practically as the cube and therefore like typical volume curves, the cerebellum volume is growing at a rate approaching the ninth power of the body length. The percentage increment of the cerebellum volume both when calculated for 5-cm. C H intervals (fig. 26, curve VII) or for age in fetal months (fig. 28, curve IV), also demonstrates a specific type of growth and shows the great increment of the part in the later fetal months as indicated by other measurements of cerebellum growth.

Compound growth. Compound growth indicates a summation of two or all types of growth in the central nervous system. It is typified by the volume and weight of the encephalon and the central nervous system volume. It is dominated by the growth of the cerebrum, since the cerebrum ranges between 85 and 95 per cent of the encephalon throughout fetal life. The rates of growth of the compound growth curves, as shown by percentage increments calculated for 5-cm. intervals (figs. 26, curves I, II, and III) and for fetal months (fig. 28, curves VI, VII and VIII), approximate the growth rate of the cerebrum. However, it will be noticed that in the early fetal months the rate of growth is not quite so high, due to the slow development of the other brain parts at this period.

The volumetric formulae (of the type here presented) of the central nervous system, the entire brain volume, and the en-

cephalon weight require a third constant in their expression, while the volume of the cerebral hemispheres can be expressed mathematically with two constants. The general empirical formula of compound growth can be expressed:

$$Y = (aX)^b + c$$

in which Y is the volume of the encephalon or entire central nervous system in cc., X is the crown-heel length in cm., and a , b , and c are constants separately determined for the entire brain volume and the central nervous system volume.

The significance of the relation between lineal and volumetric dimensions in the growth of the parts of the brain. The classification of the types of growth of the central nervous system is shown by both the volume and linear curves. For instance, both the volume curve of the cerebellum and the linear measurements of the vermis cerebelli length and the vermis cerebelli height indicate the characteristic cerebellum type of growth. It is logical that there should be a definite relation between the linear and the volume measurements of a brain part. It is also evident that the length of a brain part when it is cubed should form a curve identical in type and constant in relationship to the corresponding volume curve, provided that no additional factor, other than that which influences the growth in the linear dimensions, influences the growth in volume. Such a relationship actually exists between the formulae of the linear cerebellum curves and the formula of the cerebellum curve of volume. The formula for the cerebellum length when cubed is practically identical to the formula of the cerebellum volume. This relationship is seen somewhat better when those values are based on the cube of the body length as modified by three constants rather than by a formula based on the body length raised to a fractional exponent. The first formula is expressed below:

$$\text{Cerebellum volume (cc.)} = [0.01 (0.073 \text{ Vermis cerebelli length (cm.)} + 2.85)^3]^3$$

Likewise, the formula of the cerebellum height when cubed and multiplied by the constant 2.13 is practically identical with the formula of the cerebellum volume:

$$\text{Cerebellum volume (cc.)} + [0.01 (0.07 \text{ Vermis cerebelli} \\ \text{height (cm.)} = 2.45)^3]^3$$

These indicate that the cerebellum volume is growing at relatively the same rate as are its vertical and horizontal diameters. The growth of the cerebellum must be controlled, therefore, by factors which act alike upon the entire cerebellum.

The formulae of the fronto-occipital length and the temporal diameter when cubed bear no relation to the cerebrum volume which may be expressed by a simple constant, and the same holds true of the spinal cord length when cubed. It is obvious, therefore, that in all other brain parts except the cerebellum there must be more than one factor influencing growth which act differently on the volumetric and linear growth.

The per cent of the brain parts and the spinal cord of the encephalon. The per cent which the various brain parts and the spinal cord form of the encephalon at each fetal month can be observed by a glance at table 40 and at figures 7, 11, 15, 18, 22, and 38. Table 40 tabulates the per cent which a respective volume of a brain part or of the spinal cord forms of the encephalon. Figures 7, 11, 15, 18, and 22 demonstrate graphically the per cent which the pons and medulla, midbrain, and the spinal cord, respectively, form of the encephalon volume. Figure 38 represents a series of composite midsagittal sections which are based upon average measurements and which represent therefore a typical fetal brain for the 5-cm. C. H. interval indicated. For purpose of comparison, a magnification of each individual figure was used. To obtain this magnification, the ratio of the respective fronto-occipital diameters to the fronto-occipital diameter of the newborn was used. The detailed results of both figures and the table have been presented in the body of this paper. Taken in toto, the evidence shows conclusively: first, the high percentages which the pons and medulla, midbrain, and the spinal cord volumes form of the encephalon volume from the second to the fifth fetal month; second, the increased percentages which the cerebellum volume forms of the encephalon volume in the last three fetal months, and, third, the predominating percentages which the

volume of the cerebral hemispheres form of the encephalon volume at all times, rising to its maximum per cent (94.2 per cent) in the sixth fetal month.

SUMMARY

I. The growth of the central nervous system in the fetal period is similar in general character to the growth of the other viscera and of the major parts of the body in this period.

II. An analysis of data on the volume and dimensions of the central nervous system in the fetal period shows four distinct subtypes or varieties of growth. These are:

1. The cerebral subtype, which is characterized by, *a*) a steady and relatively slow increase in volume from the second to the beginning of the sixth fetal month and a constant and more rapid increase from this time to birth, and, *b*) by a steady and constant growth in linear dimensions from the second fetal month to birth.

2. The brain stem and cord subtype, which shows a much more rapid growth from the second to the end of the fifth fetal month than it does in the last five months of fetal life.

3. The cerebellum subtype, which proceeds very slowly from the second to the end of the fifth fetal month and then increases tremendously from the sixth month to birth.

4. The compound subtype, which represents the combined effect of two or three or all of the above varieties, predominated by the cerebral subtype.

III. The factors which control the various subtypes of growth in the central nervous system may influence the volumetric and linear determinations of a respective brain part in two ways: 1) the factors may act differently upon the volumetric and linear values as they do in the case of the cerebral growth, brain stem, and cord growth, and in compound growth, or, 2) they may act in the same manner as they do in the case of the cerebellum subtype of growth.

IV. An estimation of the percentages which the cerebrum, cerebellum, and the brain stem form of the encephalon at the various periods of fetal life offers a means of comparison of the

relative growth of these parts. The percentages which the brain stem and the spinal cord form of the encephalon are relatively high from the second to the fifth fetal month; the per cent which the cerebellum form of the encephalon is at its height in the last three fetal months, while the per cent which the hemispheres form of the encephalon reaches its maximum in the sixth fetal month.

V. The four subtypes of growth of the central nervous system may be clearly expressed by a classification of the empirical formulae of the growth curves of the various parts as follows:

1. *Cerebral growth:*

a. General linear formula: $Y = aX \pm b$.

b. Specific linear formulae:

a'. Fronto-occipital diameter: $Y = 0.175 X + 0.25$.

b'. Temporal diameter: $Y = 0.138 X + 0.3$.

c. General volumetric formula: $Y = (aX)^b$.

d. Specific volumetric formulae:

a'. Right hemisphere volume: $Y = (0.1 X)^{3.13}$.

b'. Left hemisphere volume: $Y = (0.105 X)^{3.04}$.

c'. Both hemisphere volume: $Y = (0.12 X)^{3.19}$.

2. *Brain stem and cord growth:*

a. General linear formula: $Y = (aX)^b - c$.

b. Specific linear formulae:

a'. Fronto-spinal length: $Y = (2.0 X)^{.47} - 2.2$.

b'. Spinal cord length: $Y = (10.0 X)^{.467} - 4.0$.

c'. Brain cord length: $Y = (10.0 X)^{.514} - 4.0$.

c. General volumetric formula: $Y = 0.01 [(aX)^b + c]$.

d. Specific volumetric formulae:

a'. Spinal cord volume: $Y = 0.01 [(0.17 X)^{2.57} + 11.0]$.

b'. Pons and medulla volume: $Y = 0.01 [(0.2 X)^{2.67} + 20.0]$.

c'. Midbrain volume: $Y = 0.01 [(0.168 X)^{2.56} + 12.0]$.

3. *Cerebellum growth:*

a. General linear formula: $Y = 0.01 [X^a + b]$.

b. Specific linear formulae:

a'. Vermis cerebelli length: $Y = 0.01 [X^{1.41} + 0.15]$.

b'. Vermis cerebelli height: $Y = 0.01 [X^{1.37}]$.

c. General volumetric formula: $Y = 0.01 [(aX)^b + c]$.

d. Specific volumetric formula:

a'. Cerebellum volume: $Y = 0.01 [(0.095 X)^{4.9} + 20.0]$.

4. *Compound growth:*

a. General volumetric formula: $Y = (aX)^b + c$.

b. Specific volumetric formulae:

a'. Encephalon volume: $Y = (0.125 X)^{3.16} + 1.5$.

b'. Brain and spinal cord volume:

$$Y = (0.114 X)^{3.34} + 2.0.$$

c'. Encephalon weight by literature:

$$Y = (0.13 X)^{3.19} + 1.0.$$

(In all of the above formulae, Y is the volume or length of the part under consideration (in cm. or cc.), X is the crown-heel or total length of the body in cm., and a , b , and c are empirically determined constants.)

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Resumen por los autores S. R. Detwiler y Henry Laurens

Estudios sobre la retina.

Histogénesis de las células visuales en *Amblystoma*

El estado inicial del desarrollo de la célula visual es la producción de una yema protoplásmica y un glóbulo acromático claro en las células de la capa nuclear externa. El glóbulo, que se transforma en el paraboloide del segmento interno, parece ser de origen citoplásmico y no de origen nuclear, según ha indicado Cameron. En los estados tempranos del desarrollo de las células visuales pueden percibirse masas de gránulos fuertemente teñidos en las preparaciones teñidas por la hematoxilina férrica. Estos gránulos contribuyen principalmente a la formación del elipsoide del segmento interno y del material granular del externo. En nuestras preparaciones no existe prueba alguna que indique su origen como productos del pigmento ingerido de la capa epitelial conforme supone Cameron. Pueden representar productos de la transformación de las mitocondrias.

En los estados tempranos del desarrollo las células visuales son todas ellas cónicas, coincidiendo de este modo con las condiciones características de la retina de los anfibios en vías de desarrollo (Cameron y Bernard). Los bastones y los conos se desarrollan más tarde a expensas de las células visuales primitivas no especializadas. Los bastones no aparecen en su forma definitiva hasta un periodo relativamente tardío. Las dos clases de elementos visuales cónicos son consideradas como células visuales de especialización baja las cuales se desarrollan, respectivamente, en los conos y bastones característicos mediante diferenciación divergente. Esta interpretación no apoya la hipótesis enunciada por Cameron. Los dobles conos aparecen temprano durante el desarrollo. La ausencia de figuras mitóticas durante este periodo indica que nacen de la fusión de elementos sencillos más bien que por división de un solo elemento.

STUDIES ON THE RETINA

HISTOGENESIS OF THE VISUAL CELLS IN AMBLYSTOMA

S. R. DETWILER AND HENRY LAURENS

*The Anatomical Laboratory, Peking Union Medical College, Peking, China, and
the Osborn Zoological Laboratory, Yale University*

THIRTEEN FIGURES

INTRODUCTION

The anatomical features of the visual cells in larval *Amblystoma* have been described and figured by Laurens and Williams ('17). According to this account, the retina in larvae of 30 mm. possesses characteristic rods and cones in the approximate proportion of four to three. In their figure (p. 76) and in figure 11 in this paper the identity of these elements is clearly displayed. The rods are quite large with typical cylindrical outer segments and pear-shaped nuclei, the latter of which are considerably attenuated on their inner pole. The cones, of which there are three kinds, are much smaller elements characterized by the possession of conical outer segments, long myoids, and more or less oval nuclei. These nuclei are more deeply situated than are those of the rods, which typically comprise the outer cell layer of the external nuclear layer, and which project through the external limiting membrane for variable distances.

In looking over some sections of the retinae of young larvae, our attention was attracted to the striking similarity of the visual cells, all of which possessed conical outer segments and oval nuclei, characteristics typical of cones. The general impression was received that in early stages of development the visual cells are all of one type (cone-like), which later differentiate into the two distinct categories of elements which characterize the fully developed retina.

From our observations several developmental possibilities were suggested: first, if all of these typical cone-like elements are to be regarded as cones, then the rods, which do not appear in their distinctive form until later, represent transformation products of cones; secondly, that some of these elements functionally represent rods even though possessing anatomical features characteristic of cones; and, thirdly, that all of these cone-shaped elements represent indifferent visual cells and the characteristic rods and cones come about by divergent differentiation of a visual cell of low specialization.

In general, the rods are regarded as the more primitive type of visual cell (Graham Kerr, '19, and Parsons, '15) and the cones are regarded as specialized rods. If this be true, their appearance in ontogenetic development might be expected to antedate that of the cones—a condition just opposite to that which is suggested in the developing retina under consideration. The idea that cones represent specialized rods does not seem borne out by the condition found in many reptiles in which only cones are present (Detwiler, '16). If they do represent specialized rods, then the early developing visual cells in pure cone retinæ should have to be regarded as rods—a condition which seems very unlikely.

According to Leboucq ('09), Magitot ('10) and Seefelder ('10), the two kinds of visual cells in the human retina develop simultaneously, and are mainly distinguishable by the fact that the axis of the diplosome (the centrosomes) is perpendicular to the long axis of the cone and parallel to the long axis of the rod. This is not entirely borne out in Seefelder's illustration which is reproduced in figure 12. The cones would also seem to be distinguishable by their much larger size. Cajal ('11) says that cones and rods evolve in essentially the same manner and it is difficult to distinguish them at the beginning (see also Fürst, '04).

Bernard ('03) and Cameron ('05 and '11), who studied the developing retina in amphibia, conclude that cones represent early stages in the formation of rods. Their conclusions are based on the fact that in early stages of the developing retina only conical elements are to be seen, while in later stages typically shaped rods predominate.

The difficulty in accepting the conclusions of Cameron and Bernard lies in the fact that the cone-like elements in the early stages, which they call cones, may functionally represent rods even though possessing the anatomical configuration characteristic of cones. The variability in the shape of the visual cells has made absolute rod and cone distinction difficult in some cases. We have already referred to this difficulty in an earlier paper (Laurens and Detwiler, '21, p. 225).

From our first survey of the developing retina in *Amblystoma* the conditions appeared to bear out the conclusions of Cameron and Bernard. However, more critical examination has shown that, although all the elements are cone-like in early stages of development, they possess characteristic features by which they can be divided into two groups, one of which can be readily identified as the progenitors of the definitive rods, the other of the cones. It seems hardly wise to regard the forerunners of the rods as functional cones even though possessing certain cone-like features.

METHODS

Our observations were made upon the retina of *Amblystoma punctatum*. The larvae were preserved in corrosive sublimate-acetic fixing fluid at successive twenty-four-hour intervals for a period of twenty-six days following the tail-bud stage of development. From this period on, specimens were fixed at successive intervals of from three to five days. Sections were cut 8μ in thickness and stained in Ehrlich's haematoxylin and erythrosin, iron haematoxylin and eosin, and in Held's molybdic acid haematoxylin.

OBSERVATIONS

The visual elements make their initial appearance in retinae of approximately eleven days after the tail-bud stage of development.¹ They are first seen in the posterior portion of the optic cup, just above the point of entrance of the optic nerve—a region corresponding to the optical axis of the eye (fig. 1). Prior to this

¹All references to ages are based upon days after the tail-bud stage of development.

period, the nuclei of the external nuclear layer show numerous mitoses.

The first step in the formation of the visual cells is the simultaneous appearance of a protoplasmic bud and a small achromatic

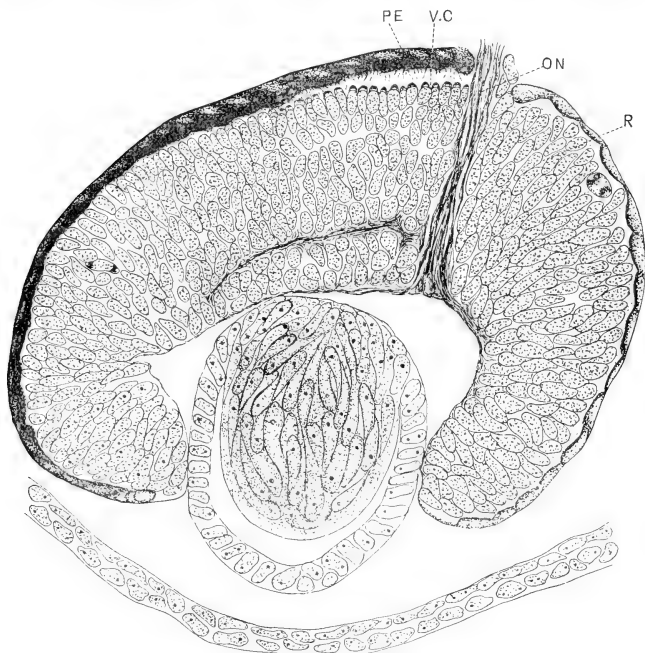


Fig. 1 Transverse section of optic cup and lens in an embryo of 11 days after the tail-bud stage of development. *ON*, optic nerve; *PE*, pigment epithelium; *R*, retina; *V.C.*, developing visual cells. $\times 275$.

globule on the pole of the nucleus applied to the external limiting membrane (fig. 2, A and B). In sections stained with iron haematoxylin, groups of deeply stained granules are usually seen just distal to the globule. The protoplasmic buds appear to be pushed through the external limiting membrane.

The conditions in retinae of forty-eight hours further development are seen in figure 3. The protoplasmic buds have become larger and assume a blunt conical shape. The globules occupy the proximal portion of the protoplasmic mass, whereas distal to them occur masses of deeply staining granules similar to the conditions shown in figure 2.

As regards the origin of the clear achromatic globule, Cameron claims that it is extruded from the nucleus. He supports his contention by observations which both he and Bernard made in



Fig. 2 Developing visual cells from the retina in an embryo of 11 days after the tail-bud stage of development. $\times 1040$.

Fig. 3 Developing visual cells from the retina of an embryo of 13 days after the tail-bud stage. $\times 1040$.

which they found a collapsed condition of the nucleus subsequent to the supposed 'sudden' discharge of its achromatic material. This view is contradicted by Leplat ('13). Graham Kerr ('19) states that the 'fatty' globule, which stains black with osmic acid, has its origin in the protoplasmic bud from the primitive visual cells.

Cameron attributes to this clear globule the power of ingesting pigment from the hexagonal pigment cells, and the intensely stained granules which he found on the summit of this globule (figs. 2 and 3) he claims to be the pigment ingested from the pig-

ment layer. Because of the fact that only the merest trace of cytoplasm can be shown to envelop the nucleus of the primitive visual cell, Cameron claims that the visual element cannot be of cytoplasmic origin, and he thus believes that it is compositely developed from the nucleus and from the ingested pigment of the epithelial layer.

It seems to us that, even if pigment ingestion can be really observed, the fact must not be overlooked that pigment ingestion could not take place unless there were cytoplasm present to ingest it. Cameron holds that the clear globule does the ingesting, but it so happens that the heavily stained granules which he saw, as well as those which we have observed, do not lie within the globule, but in the cytoplasm beyond it (fig. 3). Cameron himself remarks, ". . . and the product of the ingestion of the pigment becomes deposited upon the summit of the globule in the form of a substance which stains intensely with iron-alum-haematoxylin. In this manner, layer after layer becomes superimposed until all the visual elements without exception now assume the shape of cones, with the achromatic globules, much reduced in size, situated in their bases."

From our observations the origin of the globule cannot be stated decisively, but whenever it is present, it is always surrounded by cytoplasm and the two seem to appear simultaneously. In many initial developing cases, cytoplasmic buds can be seen in which there is no globule and yet they contain the heavy granules which are shown in figures 2 and 3. That these granules should represent products of epithelial pigment seems very doubtful from our observations. If they are ingested under the chemotactic influence of the globule, as Cameron says, then visual cell buds which lack the globule should be devoid of this deeply staining substance. Moreover, the pigment needles of the hexagonal cells are not stained nearly so heavily as are those in the visual cell buds. The former are brownish and only appear black when heavily massed. The granules in the visual cell bud are jet black in our preparations, even when appearing singly. Our observations suggest that the heavily stained granules are elaborated in the cytoplasm of the visual cell and that the globule

comes about through partial vacuolization of the cytoplasm. Occasionally, small refractive granules can be seen in the clear globule. These may represent ingested pigment, but the heavily stained ones situated distal to the globule and which comprise a large part of the visual cell, certainly give no indication of originating from the pigment epithelium.

Leplat ('13) has investigated the rôle of mitochondria in connection with the development of the rods and cones in the chick. He also found deeply stained granules in the initial protoplasmic buds. These granules, which he has called mitochondria, are to be seen in figure 13A, which is a reproduction of one of his illustrations. They are seen to bear a striking resemblance to those which we have observed (figs. 2 and 3), yet our preparations were fixed with the aid of 5 per cent acetic acid, which is a mitochondrial solvent. Leplat regards the transverse striation of the external segment and its cleavage into discs (fig. 13 B), as the expression of its mode of construction from mitochondria. In our preparations a similar transverse arrangement of the material is readily seen (fig. 5), though they cannot be regarded as mitochondrial granules for reasons previously stated. They may, however, represent chemical transformation products of mitochondria with the retention of the plan of the mitochondrial arrangement.

No evidence of rod and cone identity based on the posture of the diplosome could be gathered from our preparations. In iron-haematoxylin stained sections, the protoplasmic buds exhibited only large groups of granules as seen in figures 2 and 3. If a typical diplosome is present, such as is shown in Seefelder's illustration of the developing human retina (fig. 12) and Leboucq ('09) and Fürst ('04), it is entirely masked by other granules.

Leboucq and Seefelder describe the cone bud as being much stouter than that of the rod (fig. 12). This figure also shows that the nuclei of the cones lie in a plane closer to the external limiting membrane than the rod nuclei. In *Amblystoma* the initial protoplasmic buds are approximately of the same size. The visual elements in a retina of several days' further development show marked differences in size (fig. 4). This relationship remains

unchanged, and when the retina is fully differentiated the rod nuclei are seen to constitute the outer row of the external nuclear layer. The same relationship between the position of the rod and cone nuclei was found in the eyes of the alligator (Laurens and Detwiler, '21). In the human retina the cone nuclei occupy the more superficial position.

In retinæ of fifteen days the visual cells typically show two groups of granules (fig. 4), between which can be seen a clear re-

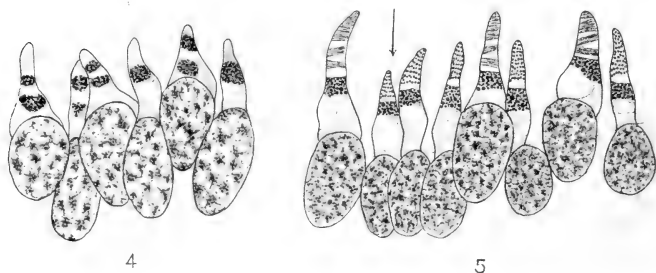


Fig. 4 Developing visual cells from the retina of a larva of 15 days following the tail-bud stage of development. The larger elements are the progenitors of the rods; the smaller, of the cones. $\times 1040$.

Fig. 5 Visual cells from the retina of a larva of 17 days following the tail-bud stage of development. The three large conical-shaped elements are differentiating rods. Their nuclei lie partly beyond the external limiting membrane. The granular material of their outer segments shows a lamellar arrangement characteristic of the fully differentiated rod (fig. 11). Arrow indicates double cone. $\times 1040$.

fractive disc marking the position of the oil drop. The original clear globule is retained in the proximal portion of the visual element and apparently develops the paraboloid. The proximal group of granules develops into the ellipsoid portion of the inner segment. The distal group of granules, as far as can be ascertained, becomes transformed into the typical granular discs which characterize the outer segment particularly in the rod. How this group of granules develops is uncertain from our preparations. That they may be separated off from the original

group by liquefaction is suggested by observations which show that in the center of the single group of granules a clear area (granule-free) can be seen as shown in figure 3. Whether or not this is actually the case, cannot be definitely determined. At any rate, the clear refractive oil drop or disc which separates these two groups of granules gives no indication of coming from the original globule, which persists in the proximal portion of the inner segment as the paraboloid.

Double cones can be seen in early stages of development, as illustrated in figure 5. These double elements give no indications as having arisen by division of single elements. When they are first observed, each member possesses its own nucleus. The total absence of mitotic figures at this stage when double cones first make their appearance suggests that they result from the fusion of preexisting single elements rather than by division.

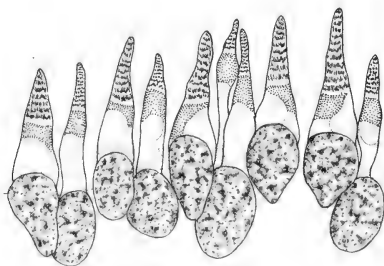
In the retina of seventeen days (fig. 5), the inner and outer segments of the visual cells are readily distinguishable. The inner group of granules has enlarged and is more deeply stained. They constitute the ellipsoid portion of the inner segment. The granular material of the outer segment of the large conical visual cells has undergone cleavage into discs which are separated by clear non-stainable bands. The lamellar arrangement of this granular material is quite prominent in the outer segment of the large visual cells, but less so in the smaller ones (fig. 5). The difference in the structural arrangement of the material in the outer segment which can be observed at this period serves as another index that the individuality of the larger conical-shaped elements is different from that of the smaller ones. Although these large elements are conical in shape, the structural arrangement of the material in the outer segment is quite similar to that which characterizes the fully differentiated rod, and all the evidence suggests that these large elements are not to be regarded as cones.

In embryos of twenty-one days (fig. 6) the visual cells show increased growth. The nuclei of the large conical-shaped elements (differentiating rods) begin to assume a pear-shape—a condition which, added to their more external situation, readily

distinguishes them from the cone nuclei. The latter are oval in shape and occupy a deeper level.

A count of the two kinds of visual cells shows that the number of larger elements slightly exceeds that of the smaller. The ratio is approximately the same as the rod-cone ratio in the fully differentiated retina, viz., four to three.

Successive stages in the development of the retinal visual cells are shown in figures 7, 8, 9, 10, and 11. These figures show the developmental conditions at twenty-six, thirty-one, thirty-six,



6

Fig. 6 Visual cells from the retina of an embryo of 21 days after the tail-bud stage of development. The nuclei of the differentiating rods are being drawn out on their inner pole, (cf. fig. 11). The cone nuclei are more deeply situated and are oval in shape. $\times 1040$.

forty, and fifty days, respectively. The difference in size between the two types of cells gradually increases as development proceeds. The large conical element gradually becomes more and more rod-shaped, and the final typical rod form which it acquires is illustrated in figure 11. Throughout these successive stages, the nucleus of this visual cell (rod) not only becomes more and more drawn out on the inner pole, but it gradually increases in its stainability, as is shown in figures 10 and 11. These nuclei extend for variable distances beyond the external limiting membrane, whereas the cone nuclei lie beneath it. The outer segment of this element shows throughout a disc-like arrangement of its granular material.

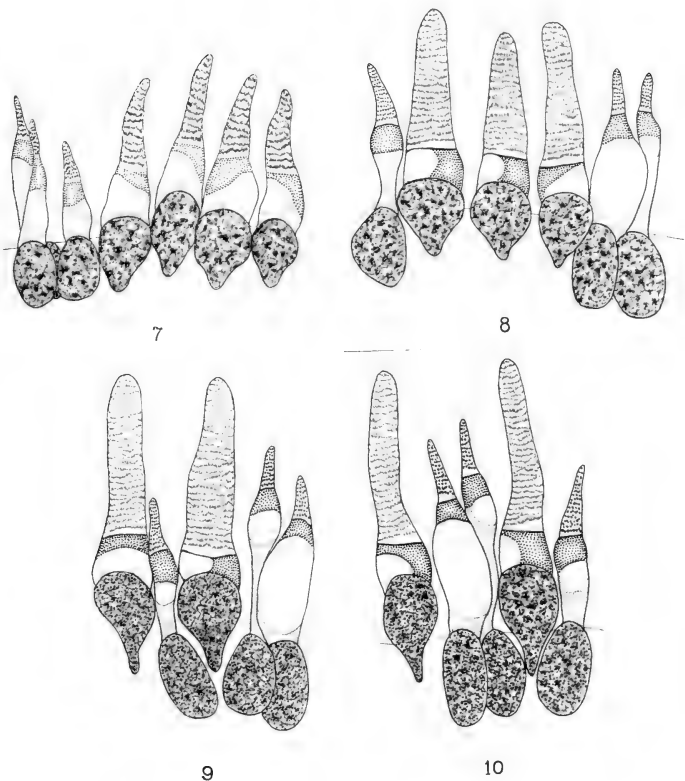


Fig. 7 Double cone, single cone, and four rods from the retina of a larva of 26 days following tail-bud stage. $\times 1040$.

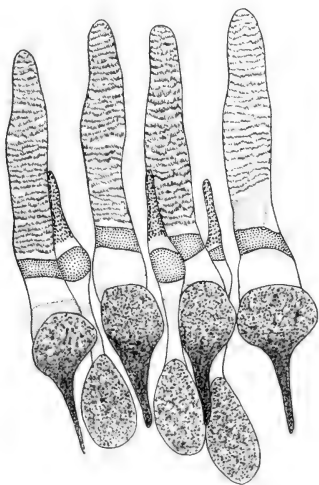
Fig. 8 Double cone, single cone, and three rods from the retina of a larva of 31 days after tail-bud stage. $\times 1040$.

Fig. 9 Double cone, single cone, and two rods from the retina of a larva of 36 days following tail-bud stage of development. $\times 1040$.

Fig. 10 Double cone, single cone and two rods from the retina of a larva of 40 days following tail-bud stage of development. $\times 1040$.

In the fully formed rod the myoid is exceedingly short. The presence of a narrow refractive disc between the outer and inner segments is demonstrable in most of the rods. In others it appears to be lacking.

Owing to the greater growth of the rods as development proceeds, the difference in size between the cones and the rods in the fully differentiated retina (fig. 11) is greater than in earlier



11

Fig. 11 Four rods and three single cones from retina of larva of 50 days after tail-bud stage. $\times 1040$.

stages of development (fig. 8). The final form of the cones is seen in figure 11. Their shape and structure agree with the description given by Laurens and Williams ('17). The outer segments are bluntly conical, and the granular material is not so characteristically arranged in lamellae as in the rods. As regards the inner segment, the size of the paraboloid is variable and is present in single as well as in both members of the double cones.

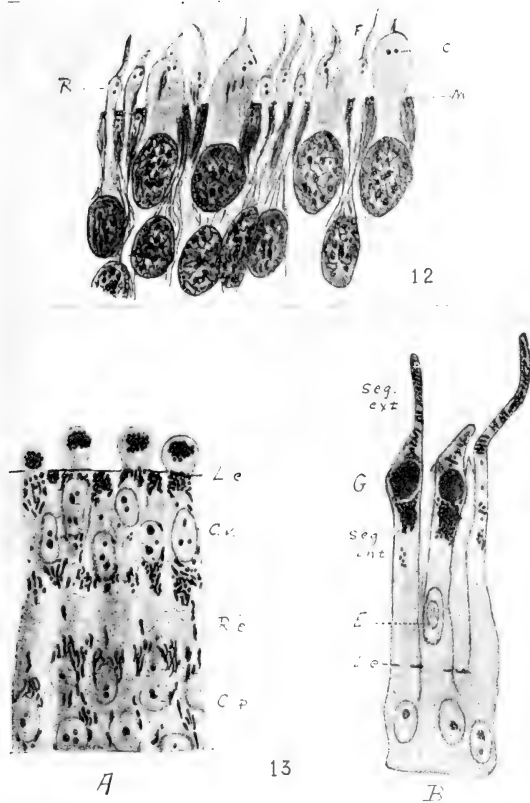


Fig. 12 Developing rod and cone visual cells, from the retina of a 345-mm. (six months) human fetus. *M*, diplosome in a Müller's fiber at the level of the external limiting membrane; *C*, diplosome in a cone cell; *F*, fiber growing out from the cone diplosome; *R*, diplosome in a rod visual cell. $\times 1000$. (After Seefelder. From Jordan and Ferguson's Text-book of Histology.)

Fig. 13 Two early stages in the development of the rod and cone visual cells in the chick. *A*, from twelve-day embryo, showing the visual cell-buds containing mitochondria. *L.e.*, external limiting membrane; *C.v.*, visual cell; *R.e.*, external reticular layer; *C.p.*, bipolar cells. *B*, from one-day-old chick, showing two complete rods and one cone, both elements containing a large lipoid spherule (*G*) and mitochondria. The cone contains an ellipsoid (*E*); *seg. int.*, internal segment; *seg. ext.*, external segment. $\times 2000$. (After Leplat. From Jordan and Ferguson's Text-book of Histology.)

The character of the connections of the visual cells with the internal nuclear layer could not be determined. In preparations stained with Held's molybdic acid haematoxylin, the internal prolongation of the rod nuclei could be followed for a considerable distance, but its final connection could not be seen.

With the establishment of the character of the centripetal connections of the visual cells which remains to be brought out by impregnation or other methods, in addition to observations on the functional behavior prior to the period when typical rods first appear, more light may be thrown upon the identity of the developing visual cells in Amphibia.

SUMMARY

1. The fully differentiated retina of *Amblystoma punctatum* possesses characteristic rods and cones (fig. 11).

2. The initial stage in the development of the visual cell is the production of a protoplasmic bud and a clear achromatic globule from the cells of the external nuclear layer (fig. 2). The globule, which becomes the paraboloid of the inner segment, appears to be of cytoplasmic origin, and not of nuclear, as asserted by Cameron.

3. In early stages of the development of the visual cells (eleven to thirteen days after tail-bud stage) masses of deeply staining granules are seen in iron-haematoxylin preparations (figs. 2 and 3). These granules contribute mainly to the formation of the ellipsoid of the inner segment and to the granular material of the outer segment. There is no evidence from our preparations which indicates that they are products of pigment ingested from the epithelial layer. They may represent transformation products of mitochondria.

4. In these early stages the visual cells are all cone-like (figs. 2 and 3), agreeing in this respect with the conditions characteristic of the developing amphibian retina (Cameron and Bernard). Rod and cone identity based on the position of the diplosome such as has been described by Fürst, Leboucq, and Seefelder has not been demonstrated. Rods and cones are later differentiated from the primitive, non-specialized visual cells.

5. Rods do not appear in their definitive form until relatively late in development. Their progenitors consist of large conical-shaped elements which possess, in early stages of development (fifteen days), an individuality by which they can be distinguished from the true cones. These elements are larger, their nuclei occupy a more external position than that of the cones, and the granular material of their outer segments becomes arranged in lamellae characteristic of the fully differentiated rod (figs. 5 and 6). The ratio of these conical-shaped rod progenitors to that of the cones is approximately the same as that of the rod-cone ratio in the fully differentiated retina (*viz.*, four to three).

6. The two kinds of conical-shaped visual elements as seen in figure 4 (large and small) are regarded as visual cells of low specialization, which develop, respectively, into characteristic rods and cones by divergent differentiation (fig. 11). This interpretation is not in support of the view advanced by Cameron, who regards all of the conical-shaped visual cells in early stages of amphibian development as cones and the later appearing rods as transformed cones.

7. Double cones appear very early in development (fig. 5). The absence of mitotic figures in the nuclei of the external nuclear layer during the period when double cones first appear suggests that they are formed from the fusion of single elements rather than from division.

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Resumen por los autores, O. Larsell y M. L. Mason

Degeneración experimental del nervio vago y su relación con las terminaciones nerviosas del pulmón del conejo

La degeneración experimental del nervio vago del conejo, seguida de tñido intravital de los pulmones con el azul de metileno, conduce a las siguientes conclusiones: Los nervios vagos suministran las fibras pregangliónicas que terminan en las células ganglionares intrapulmonares, diseminadas a lo largo de los bronquios y sus ramas, como demuestra la desaparición de la mayor parte de las redes pericelulares alrededor de dichas células después de dejar pasar suficiente tiempo para que degeneren las fibras del vago del mismo lado. La mayor parte de estas fibras pregangliónicas provienen del vago del lado homolateral, pero algunas de las redes pericelulares persisten en el pulmón después de la degeneración del vago del mismo lado. Estas redes derivan parcialmente, con toda probabilidad, del lado contralateral mediante entrecruzamiento de las fibras en el plexo pulmonar posterior.

El número de fibras de la musculatura bronquial que aparecen teñidas varía muy poco o nada al degenerar las fibras del nervio vago. Después de la degeneración de dicho nervio persisten en el pulmón unas pocas terminaciones sensoriales, en el lado operado. Estas terminaciones se cree provienen en parte del vago del lado opuesto. El número de fibras nerviosas que se tiñen en las paredes de los vasos sanguíneos pulmonares no es afectado por la degeneración del nervio vago.

Translation by José F. Nonidez
Cornell Medical College, New York

EXPERIMENTAL DEGENERATION OF THE VAGUS NERVE AND ITS RELATION TO THE NERVE TERMINATIONS IN THE LUNG OF THE RABBIT¹

O. LARSELL AND M. L. MASON

FIVE FIGURES

In a recent article one of the authors (Larsell, '21) has demonstrated histologically the presence of nerve terminations in the lung of the rabbit and their distribution in relation to the various parts of the bronchial tree and of the pulmonary blood-vessels. An adaptation of the methylene-blue technique was employed which, with the normal lung, gave results that appeared sufficiently constant to warrant an attempt to repeat the method on experimental animals. The hope was entertained that it might be possible to determine the elements which belong to the vagus nerve as shown by their disappearance by degeneration, following section of the vagus nerve in the neck region.

As in the previous work, rabbits were utilized. The vagus was sectioned in the midcervical region and the cut ends were allowed to retract. Ether anaesthesia was used during the operation, and aseptic precautions were observed. Twelve animals were operated upon, but only four lived for the period of twenty-eight days which was deemed necessary for complete degeneration of the severed fibers.

It is worthy of remark that in these four rabbits the left vagus had been sectioned, while in the animals which died, apparently more or less as a result of the operation, either the right vagus or both right and left nerves had been cut. All of these animals died within seven days after the vagotomy, and were not utilized. Except that two showed tuberculous lesions

¹ Contribution from the Zoological Laboratory, Wm. A. Loey, Director, and from the Anatomical Laboratory of Northwestern University.

on autopsy, no other serious lung disturbance was noted, and they apparently died from other causes. Two of the animals which were subjected to the double vagotomy, however, died within forty-eight hours from respiratory difficulties due to closure of the glottis.

The lungs of the four animals that survived gave results which were in such accord that they are believed to be typical. In these four animals the lungs were stained and treated by the procedure described in the article to which reference has already been made. In each case both lungs from a given individual, namely, the right normal lung and the left lung from the operated side, were carried through the various steps of technique together, in order to insure uniformity of treatment. It was believed that in this way the right lung, with its nerve intact, would serve as a check on the quality of staining of the nerve fibers in the left lung, the vagus fibers to which had been severed.

A portion of the left vagus, including the neuroma at the level of the lesion, was also removed. This was fixed in 0.5 per cent osmic acid, imbedded in paraffin by the usual process, and sectioned. Transverse sections 10μ thick were made of portions from the proximal and distal ends, respectively, of the removed portion of the nerve, and longitudinal sections, mounted in series, were made of the remainder, including the neuroma.

The corresponding portion of the right vagus was also removed from one animal, fixed and stained in osmic acid, imbedded and sectioned transversely. It showed no indication of difference from the proximal end of the left vagus which was taken from the same animal (cf. fig. 1). There appears, therefore, to have been no upward degeneration in the operated nerve.

All of these nerves which had been subjected to operative procedure showed degeneration of the myelin sheaths below the level of section (fig. 2). In one animal (R7) there appeared a few rings in the distal part of the nerve (fig. 2, *my.*) which bore the semblance of normal myelin sheaths, as observed in transverse section, but these could not be followed consecutively through the series of sections, and probably represent only short stretches of myelin sheaths which had not yet completely

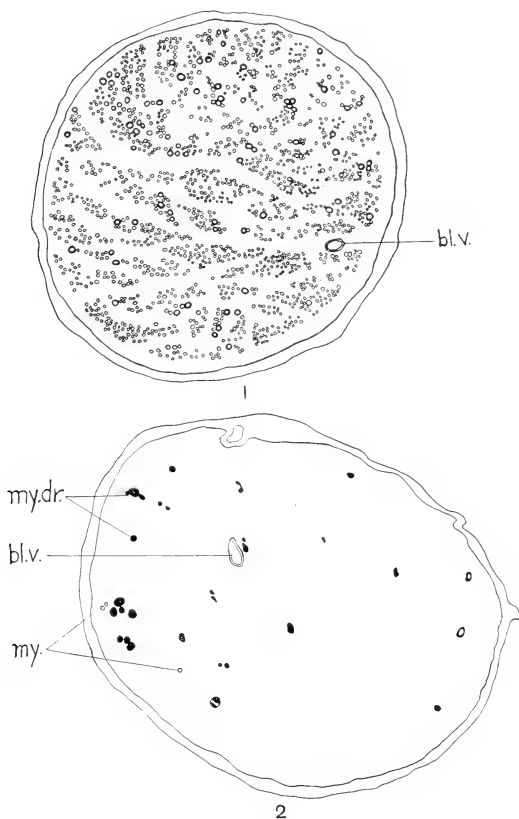


Fig. 1 Transverse section of left vagus nerve of rabbit (R7) which had been vagotomized twenty-eight days prior to killing of the animal. The section represents a portion of the nerve proximal to the level of the lesion, showing the myelinated fibers of various sizes in apparently normal condition. *bl.v.*, blood vessel. Osmic acid stain. 10μ . $\times 85$.

Fig. 2 Transverse section of same nerve distal to level of lesion, showing complete degeneration of the myelinated nerve fibers, with the possible exception of those marked *my.* *bl.v.*, blood vessel; *my. dr.*, droplets of degenerated myelin. Osmic acid stain. 10μ . $\times 85$.

broken up into the characteristic myelin droplets (fig. 2, *my. dr.*) of degenerated nerves. In the nerves from the other three animals (R8, R9, and R14) degeneration of the distal portion of the cut nerve was undoubtedly complete.

Since the preparations were stained by the methylene-blue technique, the degeneration of fibers within the lungs themselves could only be inferred from the fact that a relatively very small number of fibers were stained in the main nerve trunks of the left lung. The corresponding nerve bundles of the right (normal) lung were very well stained in three of the animals, but very poorly in the fourth. The left lung of this animal (R14) gave

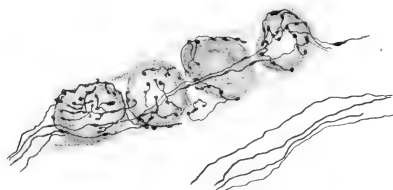


Fig. 3 A cluster of four ganglion cells within the right lung of a rabbit (R7) whose left vagus had been sectioned and degenerated. The pericellular networks about the cells have the normal appearance. Methylene-blue stain. 60μ . $\times 600$.

results comparable with those obtained from the corresponding lungs of rabbits R7, R8, and R9. The right lung of R14 was not well injected with the stain because of blocking of its pulmonary artery, so that a sufficient quantity of the stain did not have access to the proper regions of the lung to insure a good stain of the nerve fibers and terminations.

Study of the intrapulmonary ganglia of the right lung in the three well-stained cases revealed an apparently normal picture. All or nearly all of the cells were surrounded by more or less complete pericellular networks (fig. 3) which represent the terminations about these cells of preganglionic fibers from the vagus nerve. An occasional cell appeared destitute of such a network,

but this condition had previously been found to obtain also in non-experimental rabbits when stained by the same method.

The left lung, however, revealed a strikingly different picture with respect to the ganglia as well as to the intrapulmonary nerve trunks. As shown in figures 4 and 5, nearly all of the cells were characterized by an absence of the synaptic network. As compared with the ganglionic cells of the right lung, the majority of nerve cells in the left lung appeared also to be somewhat shrunken in size.

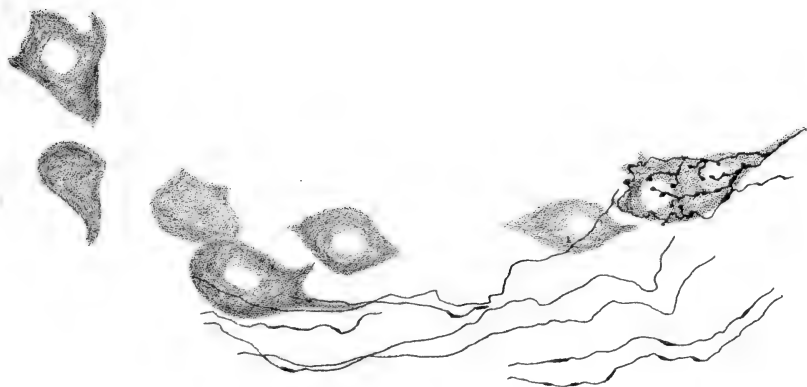


Fig. 4 Ganglion cells from left lung of same rabbit (R7) showing a single pericellular network. Methylene-blue stain. 60μ . $\times 600$.

An occasional pericellular network was found about a cell here and there in the left lung, but these were few in number. They evidently represent the terminations of preganglionic fibers from other sources than the homolateral vagus. Schiff ('50) states that each lung receives fibers from both vagi, and Möllgaard ('12) more recently has come to similar conclusions. It appears probable, therefore, that preganglionic fibers from the contralateral vagus cross through the posterior pulmonary plexus, enter the lung at the hilum and terminate around some

of the cells of the intrapulmonary ganglia. The possibility that some of these fibers may come through the sympathetic from the upper white rami is not excluded.

The nerve fibers in the smooth musculature of the bronchial tree, so far as could be determined, were stained in apparently equal numbers in both lungs and with equal intensity. This would appear to indicate that these fibers are all postganglionic, although it does not follow that all have their origin from intrapulmonary cells. Some may be axonic processes from cells of the ganglionic clusters in the posterior pulmonary plexus or elsewhere. Chase and Ranson ('14) state that the bronchial rami



Fig. 5 Ganglion cells and nerve fibers from left lung of same animal (R7). No pericellular networks are present in this cluster of cells, although fine nerve fibers, believed to be postganglionic, are stained in the immediate vicinity of the cells. A single large fiber (*s.fi.*), probably sensory, is also present. Methylene-blue stain. 60μ . $\times 600$.

of the vagus contain large numbers of myelinated fibers and take from the vagus nerve a considerable proportion of the myelinated fibers which have continued down to this level. Some of these are of large size and are undoubtedly sensory, but the majority are small and probably represent the preganglionic fibers to the lungs within the vagus nerve. The absence of any obvious degeneration of the nerve fibers to the bronchial musculature speaks against Möllgaard's statement that the bronchiolodilatator fibers take their origin from cells of the vagus ganglia.

The number of stained nerve processes in the walls of the pulmonary blood-vessels also appears to be undiminished on the

experimental side as compared with the intact side. The source of these fibers, according to Möllgaard, is the second and third thoracic ganglia of the sympathetic trunk, chiefly of the homolateral side, but also from the ganglia of the opposite side. We have nothing to add to this statement. The nerve processes of the pulmonary vessels are probably all postganglionic fibers.

No exhaustive study was made of the sections from the experimental side with reference to sensory nerve terminations. However, a few such endings were observed at some of the stations which have been found to be characteristic (Larsell, '21). Also a few large nerve fibers (fig. 5, *s. fi.*) may be followed for considerable distances along the bronchial tree in the lung of the experimental side.

These sensory terminations and the fibers of large size which probably lead to them are very likely derived, in large part, from the vagus of the opposite side, although Möllgaard, in the article previously cited, has brought forth evidence that the lung receives sensory fibers also from the second and third thoracic spinal ganglia. The physiological experiments of Barry ('13) and Roger ('17) clearly demonstrate that afferent impulses from the lung travel through the sympathetic trunk and white rami communicantes to the spinal cord. Accordingly, some of the sensory fibers and their terminations which remain in the lung after degeneration of the vagus branches leading to it may have their origin in the second and third thoracic spinal ganglia. Both Möllgaard ('12) and Molhant ('13) state that the origin of the sensory fibers which pass to the lung by way of the vagus nerve is in the body of the ganglion nodosum, well above the level at which the vagotomy was performed. Hence it is not likely that any aberrant cells from this ganglion were responsible for the sensory terminations which remained in the lung after degeneration of the lower part of the vagus leading to it.

SUMMARY AND CONCLUSIONS

The vagus nerves supply the preganglionic fibers to the intrapulmonary ganglion cells which are scattered along the bronchi and their branches, as is shown by the disappearance of most of the pericellular networks about these cells following experimental degeneration of the vagus nerve of the same side.

Most of these preganglionic fibers come from the vagus of the homolateral side, but some pericellular networks remain in the lung after degeneration of the vagus of the same side. These are probably derived in part from the contralateral side by the crossing over of fibers through the posterior pulmonary plexus.

The number of fibers in the bronchial musculature which take the stain is affected very little, if at all, by the degeneration of the fibers of the vagus nerve.

A few sensory terminations remain in the lung of the operated side after degeneration of the vagus fibers leading to it. These endings are believed to be derived, in part, from the vagus of the opposite side.

The number of nerve fibers in the walls of the pulmonary blood-vessels which take the stain is unaffected by degeneration of the vagus nerve.

In conclusion we desire to express our thanks to Dr. S. W. Ranson for valuable suggestions in the course of the work on which the present paper is based.

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